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DEDICATORY INTRODUCTION

The appraisal of a scientist by his contemporaries is a practice still common and by methods equally unscientific. We compare ourselves among ourselves with the assurance of one measuring the yardage of cloth or weighing staple produce by the pound, yet we lack both weights and measures. Instead, we use marks for the beginner at six, credits and grade points through his adolescence, degrees during his maturity, and stars and prizes for old age.

Humanists remind us that this incompetence of scientists at human appraisal follows from the inadequacy of scientific methods and material. The essential study of mankind still is man, not cells and atoms, and human qualities, primarily intrinsic, have no counterpart in other things either animate or inanimate. Scientific tools and procedure have advanced far in the analysis of stars, vitamines, and protons, or toward manipulating the qualities of garden peas and fruit flies; but while such methods apply well enough in the quest of nature around us, they leave the nature of man quite unapproachable.

Toward these claims scientists have come to be apprehensive. They would have to admit many incompetences and deplore the inadequacy, the crudity of their techniques; but mindful of the scientist's ignorance of stars and atoms, cells and fruit flies, they would fail to overrate the humanist's knowledge of man. More than likely, they would choose to reflect on man's descent, whose lowly beginnings, very long past, and slow advancement occasion the more respect for his present competence and prospective attainments. And likely, too, they would not time man's future progress with his past performance. Were rates of human advancement fixed and equal, then scientists could as well leave their tasks for later generations. But their record of achievements has thwarted the rule of time and left unpredictable both the rate and the limits of advancing knowledge, whether of atoms, of cells, or of man.

Validity of scientific knowledge rests not upon that which is only indicated or assumed, but solely upon what can be proved and demonstrated. Thus the scientist has come to exercise diligent care both in the methods he employs and in the material which he selects. Even when human problems may be the ultimate aim, his immediate interests and approach are frequently determined by severe limitations of present-day techniques and of inadequate data. These imposed conditions have worked individual hardships, but they have also benefited science at large through discoveries of invaluable material and the development of first-rate methods. Among the best-known instances of this was the discovery of microörganisms following the invention of the microscope. The far-reaching results of the craftsmanship and findings of the quaint genius of Delft can scarcely be overestimated. It is enough, perhaps, to cite, in the wake of Leeuwenhoek's work, the contributions of Pasteur, who, with his contemporaries, established the beginnings of a new biology around the living cell as the key to all subcellular, unicellular, and multicellular forms of life.

Modern trends in biology reflect the significance of this prime discovery and formulation of the cellular nature of life phenomena. All problems of these phenomena, in their last analysis, are resolvable to problems of the living cell, which is the unit not only of the structure and functions of living things, but also of their reproduction, development, heredity, and evolution. This accounts largely for the increasing emphasis nowadays on studies involving unicellular organisms or the unicellular stages of many-celled organisms. Here all vital phenomena find their most elemental expressions so that the fundamental problems can be recognized and isolated from the incidental. Improved means of environmental control are being made to satisfy the most rigorous requirements for reproducible experimentation, thus emphasizing the distinction which biology properly enjoys as an experimental science.

This stage of experimentation in biology obviously has not been reached by any sudden flight or mutational change. It has come as a result of many decades of most painstaking critical observation and descriptive analyses in both field and laboratory-foundational work which is still far from completion. The systematist, descriptive morphologist, and ecologist have performed this indispensable rôle, for which, in modern times, they have not infrequently received inadequate recognition.

In the annals of American biology having to do especially with unicellular forms of life, one name stands out today preëminently as that of a pioneer in preparing the way for modern biological developments and as a major contributor to those developments. Subsequent to his earlier studies on cell-lineage in molluscs, he recognized many unexplored or poorly charted regions of the unicellular world, and to these much of his extensive research has since been devoted. Colonial and individual flagellates and ciliates of streams and lakes, and a prodigious number of both armored and unarmored forms of marine dinoflagellates and sessile marine ciliates were the basis, as free-living material, for various papers and voluminous, elaborately and accurately illustrated monographs. Probably his major interests and contributions, however, have had to do with protozoa of parasitic habit. These have included a variety of representatives of the four major classes, and their hosts have ranged from other protozoa and plants to coelenterates, worms, insects, crustaceans, molluscs, echinoderms, fish, amphibia, reptiles, birds, and mammals, with special interest and experimental and exhaustive studies on protozoan parasites of man. Together with this array, mention might also be made of some of his wider interests and investigations on metazoan parasites of man and of other vertebrates chiefly as further illustrative of his insatiable desire to help rid mankind of its worst scourge and enemy, disease.

Adequate appraisal of the varied and invaluable accomplishments of this American biologist must await the judgment of later generations. Those who know him best as teacher, friend, and counselor have experienced the value of personal contacts which somehow inspire and direct the course of individual careers toward the best they can give to a struggling, bewildered, searching humanity. Such contacts, however, unfit the recipients as critical evaluators of the less tangible though more durable qualities of a master's handiwork. They reckon well enough the spiritual counterpart which integrates the intellectual with all the other attributes comprising the man, but their appraisal of the scientist would want for weights and measures that do not now exist. None lack full confidence, however, in the nature of the appraisal that will eventually be imposed.

To a born teacher and vivid inspiring lecturer; a tireless, penetrating, farseeing researcher; an efficient, exacting administrator and judicious editor; a wise counselor and faithful, obliging friend; one who understands youth and counts upon them to carry on; one to whom nature's diversities, including mankind, only betray man's spatial sketches of a deeper temporal unity,—to Professor Charles Atwood Kofoid this volume of the series to which he so abundantly contributed is dedicated as a meager token of the affection and admiration of its contributors, of hosts of classroom and graduate students, and of his colleagues.

C. V. TAYLOR,
Herzstein Professor of Biology,
Stanford University

CILIATES FROM BOS INDICUS LINN.
III

EPIDINIUM CRAWLEY, EPIPLASTRON GEN. NOV., AND OPHRYOSCOLEX STEIN

BY

C. A. KOFOID AND R. F. MacLENNAN

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CILIATES FROM BOS INDICUS LINN. III.

EPIDINIUM CRAWLEY, EPIPLASTRON GEN. NOV., AND OPHRYOSCOLEX STEIN

BY

C. A. KOFOID AND R. F. MACLENNAN

INTRODUCTION

Epidinium and *Ophryoscolex* are now clearly recognized as separate genera, although they have at times been united on the basis of similarity in their skeletal plates. Because of the clear-cut characters of these genera there is very little revisional work to be done. This paper (the last in the series dealing with the ciliates of the Indian ox) is mainly a description of species of these genera present in *Bos indicus*, with notes on the holotrichous ciliates present in this host.

ACKNOWLEDGMENTS

For grants in aid, acknowledgments are made to Regent William H. Crocker, of the University of California, whose generous support made possible the expedition of the senior author into the Orient; and to the Bache Fund of the National Academy of Sciences, whose grant provided for the preparation of the material and illustrations; in addition, acknowledgments are made to those mentioned in the first paper of this series.[1]

EPIDINIUM Crawley

Epidinium Crawley 1923, pp. 394, 401; Dogiel 1927 *partim*, pp. 156–181, figs. 90–99 (for pp. 181–183, figs. 100–102, see *Epiplastron*).

Diagnosis.—Body elongate and twisted around the main axis; dorsal membranelle zone behind the anterior end of the body; main skeletal complex composed of three plates; macronucleus a straight, elongate body; two contractile vacuoles.

Type species.—Crawley (1923) designated *Epidinium ecaudatum* (Fiorentini 1889) as the type species.

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[1] Kofoid, C. A., and MacLennan, R. F., 1930.

REVIEW OF LITERATURE

Although the genus *Epidinium* was not described by Crawley until 1923, many of the species of this genus were among the first species of Ophryoscolecidae to be described. Fiorentini (1889) described *Diplodinium ecaudatum, D. caudatum,* and *D. cattanei.* Eberlein (1895) described another species under the name *D. caudatum* which was renamed *D. eberleini* by Cunha (1914). Sharp (1914) redescribed two of Fiorentini's species and three new ones, but considered them as formas of *D. ecaudatum.* This group is separated from the rest of the species of *Diplodinium* by the shape of the body, size and position of the membranelle zones, and the number of skeletal plates. In these respects it shows closer relationships to *Ophryoscolex* than to *Diplodinium.* Cunha (1914), Awerinzew and Mutafowa (1914), and Dogiel (1925) therefore took the *D. ecaudatum* group and placed it in *Ophryoscolex.* Crawley (1923), however, showed that this group belonged neither in *Diplodinium* nor in *Ophryoscolex* and erected a new genus, *Epidinium,* for it.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The morphology of *Epidinium* is more accurately known than that of the rest of the Ophryoscolecidae, as a result chiefly of the work of Sharp (1914), Bělar (1925), and Dogiel (1927). The differences revealed between *Epidinium* and the other closely related genera clearly established the validity of this genus.

The oral membranelle zone is relatively small and inclined ventrally and to the left at a steep angle. The dorsal membranelle zone lies behind the anterior end of the body near the level of the anterior tip of the macronucleus. It arises near the dorsal edge of the skeletal complex and extends to the left for about one-third of the circumference of the body. Thus, in position and size, the dorsal zone of *Epidinium* gives a transition from this organelle as present in such genera as *Diplodinium, Ostracodinium,* and *Polyplastron* to the elongated dorsal zone found in *Ophryoscolex.*

The body is almost circular in cross-section, the slight lateral flattening being nearly imperceptible. The main longitudinal axis of the body is twisted, the anterior end of the body being bent ventrally and twisted to the right about the main axis. This twisting is particularly clearly shown in a dorsal view and involves the position of the skeletal plates, nuclei, contractile vacuoles, and spines, as well as the general contours of the body. In other genera, such as *Diplodinium, Eudiplodinium,* and *Ostracodinium,* the body shows very little, if any, twisting around the main axis.

The main skeletal complex of *Epidinium* is composed of three plates with their anterior ends lying close against the edge of the oral area. The ventral plate arises near the left ventral edge of the oral area and extends posteriorly and to the right. It terminates in the posterior third of the right ventral surface. The large median plate arises along the ventral half of the right side of the oral area and extends posteriorly parallel and adjacent to the ventral plate. The narrow dorsal plate arises along the dorsal side of the oral area and extends posteriorly between the median plate and the macronucleus. The ventral and median plates lie close together throughout their whole length. The dorsal and median plates lie close together except in the anterior third of the body where there is a small window of undifferentiated ectoplasm between the two plates. The main skeletal complex is of this form throughout the genus. One species, *E. eberleini,* has an accessory skeletal plate in the ventral spine in addition to the main complex of plates centering around the oral area. This accessory plate is a long thin structure extending from the middle of the ventral side to the tip of the ventral spine. The main skeletal complex of *Epidinium* is similar to that of *Ophryoscolex,* but does not extend into the main caudal spine, as does that of the latter genus. The presence of an accessory plate in the ventral spine of *E. eberleini* is paralleled by the presence of accessory plates in the spines of *Ophryoscolex caudatus* and other species of that genus. The similarity of the skeletal systems of *Epidinium* and *Ophryoscolex* is an important indication of their close relationship.

The most commonly used characters for specific differentiation in *Epidinium* are the number, shape, and position of caudal spines or lobes. The spines, although highly variable in size, as in the other genera of the family, are stable in number and position and are very useful for the differentiation of species. In one species the presence of an accessory skeletal plate in the ventral spine is an additional distinctive feature. The shape of the body is another important characteristic, though less obvious than spination. The body of Sharp's *E. ecaudatum* group is elongate and tapered posteriorly, while the body of *E. cattanei* Fiorentini (non *D. cattanei* Sharp) is relatively short and is abruptly truncated posteriorly. Similar differences separate *E. caudatum* and *E. hamatum,* as pointed out by Schulze (1924). All these characteristics are the same as those which have proved of most use for a specific classification in the other genera of the Ophryoscolecidae. As in the other papers of this series (Kofoid and MacLennan, 1930, 1932), we have not used the systematic unit forma.

ECAUDATUM GROUP

The six species making up this group were first assembled by Sharp (1914) as formas belonging to a single species, *Epidinium ecaudatum* (his *Diplodinium ecaudatum*). These forms, however, are morphologically distinct and there is no evidence to show that individuals of one type give rise directly to the other types in the reproductive processes. Many hosts have been found among domestic cattle which have many ciliates of only one type giving positive evidence that they reproduce true to form. Although this can be finally proved only by pure-line cultures, the weight of evidence now available shows that these forms differing in spination are true species and not mere fluctuating variations or formas.

The *Epidinium ecaudatum* group is characterized by a tapering of the posterior half of the body, which ends in the small rounded posterior end. Speciation within this series is based upon the spination of this small posterior end. There is a complete series of species ranging from *E. ecaudatum* with no spines, to *E. parvicaudatum* with five small conical spines. *E. caudatum* has a single large ventral spine. *E. bicaudatum* has a smaller dorsal spine in addition to the ventral spine. A right lateral spine is added in *E. tricaudatum*. *E. quadricaudatum* has both a right and a left lateral spine in addition to the dorsal and ventral spines. *E. parvicaudatum* has two right lateral spines, making a total of five spines for this species. The *E. ecaudatum* group is similar to the *Diplodinium anacanthum* group in this serial development of caudal spines on a relatively narrow, tapering, posterior end.

E. hamatum, E. bulbiferum, E. cattanei (Dogiel's *E. ecaudatum fasciculus*), and *E. gigas* have been included by Dogiel in the *E. ecaudatum* group, but none of these species shows the tapering posterior end of the body as do all the species which have been included by us in the *E. ecaudatum* group.

Epidinium ecaudatum (Fiorentini 1889)

Diplodinium caudatum Fiorentini 1889, pp. 15–16, pl. 3, figs. 1–2; Eberlein in 1895, pp. 263–264, fig. 19; Buisson 1923*a*, pp. 105–106; Fantham 1926, p. 568.
Diplodinium ecaudatum forma *ecaudatum* Sharp 1914, pp. 62–90, figs. A–D, pls. 3, 6, 7; Buisson 1923*a*, pp. 106–113, fig. 37; Becker and Talbott 1927, p. 354, fig. 22.
Ophryoscolex inermis Cunha 1914*a*, p. 4; 1914*b*, pp. 58, 60–62.
Ophryoscolex labiatus Awerinzew and Mutafowa 1914, pp. 114–115, fig. 6.
Ophryoscolex ecaudatus Dogiel 1925, pp. 135, 192, 248, 250.
Ophryoscolex ecaudatus forma *ecaudatus* Dogiel 1925*c*, pp. 57–61, figs. 18–20.
Epidinium ecaudatum Crawley 1923.
Epidinium ecaudatum forma *ecaudatum* Dogiel 1927, pp. 159–161, fig. 90; 1932, p. 97.

Diagnosis.—Body relatively long (2.3–2.9 dorsoventral diameters in length) and tapered posteriorly; smoothly rounded posteriorly. Length 90–152μ.

Occurrence.—*E. ecaudatum* has been reported from domestic cattle from Italy by Fiorentini (1889), from Germany by Eberlein (1895), from California by Sharp (1914), from Iowa by Becker and Talbott (1927), from Brazil by Cunha (1914), from the U.S.S.R. by Dogiel (1927), and from Uganda by Dogiel (1932). It has also been reported from reindeer (*Rangifer tarandus*) from northern U.S.S.R. by Dogiel (1925), and from antelope (*Tragelaphus scriptus*) from Uganda by Dogiel (1932).

Relationships.—E. *ecaudatum* is the simplest member of the *E. ecaudatum* group.

Epidinium caudatum (Fiorentini 1889)

Plate 1, figure 1; figure A, 1–2

Diplodinium caudatum Fiorentini 1889, p. 24, pl. 3, fig. 2; non Eberlein 1895, pp. 260–261, pl. 17, fig. 16; Cunha 1914*a*, p. 31; 1914*b*, p. 62; Buisson 1923*a*, p. 119; 1923*b*, p. 158; Schulze 1924, p. 656; Becker and Talbott 1927, p. 354.

Diplodinium ecaudatum forma *caudatum* Sharp 1914, pp. 90–91, pl. 5, fig. 6; Buisson 1923*a*, pp. 113–117, fig. 39; Becker and Talbott 1927, pp. 354–355, pl. 3, fig. 25.

Ophryoscolex intermixtus Awerinzew and Mutafowa 1914, pp. 112–113, fig. 3.

Ophryoscolex inermis var. *caudatus* Cunha 1914, p. 113, fig. 40.

Ophryoscolex ecaudatus forma *caudatus* Dogiel 1925*b*, pp. 137, 141; 1925*c*, pp. 57–59; Dogiel and Fedorowa 1925, p. 102, fig. 6.

Epidinium caudatum Crawley 1923, p. 412, pl. 29, figs. D4–D6; Dogiel 1927, p. 269.

Epidinium ecaudatum forma *caudatum* Dogiel 1927, pp. 161–163, fig. 91*a-c;* 1932, p. 97, 98.

Diagnosis.—Body elongate (2.04–2.86 dorso-ventral diameters in length) and tapered posteriorly; single ventral caudal spine. Length 85–140μ.

Description.—The body is elongate (2.04–2.86 dorsoventral diameters in length), and tapering toward the posterior end. The ventral and left surfaces are flat or slightly concave, the dorsal and right surfaces strongly convex. The oral area (0.37–0.41 dorso-ventral diameter in diameter) is inclined ventrally at an angle of about 45° and to the left at an angle of about 30°.

The single caudal spine (0.38–1.19 dorso-ventral diameters in length) arises from the postero-ventral end of the body. It curves dorsally and to the right. In one specimen the caudal spine was bifurcate.

Fine longitudinal striations cover the cuticle. Beneath the cuticle three skeletal plates extend from the operculum and the right side of the oral area posteriorly past the middle of the body. The ectoplasm is relatively thin except in the anterior end of the body in the region of the locomotor organelles.

The macronucleus is an elongate body (0.88–1.72 dorso-ventral diameters in length) lying beneath the right dorsal surface adjacent to the edge of the dorsal skeletal plate. The micronucleus is a small ellipsoidal body 3–7μ in diameter. It lies in a small depression in the left dorsal side of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface, to the right of the dorsal mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly from the mouth and toward the right surface. The fibrils lining it are fine and difficult to see except in sections. The boundary layer is well developed. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a narrow ectoplasmic structure in the postero-ventral end of the body. It is lined by fine, longitudinal fibrils. The anus is a narrow, elliptical opening near the right dorsal side of the base of the ventral spine.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from 10 specimens selected at random from *Bos indicus*.

Axis	Microns	Proportional
Length	107 (85–140)	2.43 (2.04–2.86)
Transdiameter	41 (36–51)	0.95 (0.90–1.00)
Dorso-ventral diameter	44 (37– 54)	1.00
Macronucleus	53 (36– 88)	1.19 (0.88–1.72)
Oral area	20 (18– 21)	0.45 (0.37–0.41)
Caudal spine	25 (15– 50)	0.57 (0.38–1.19)

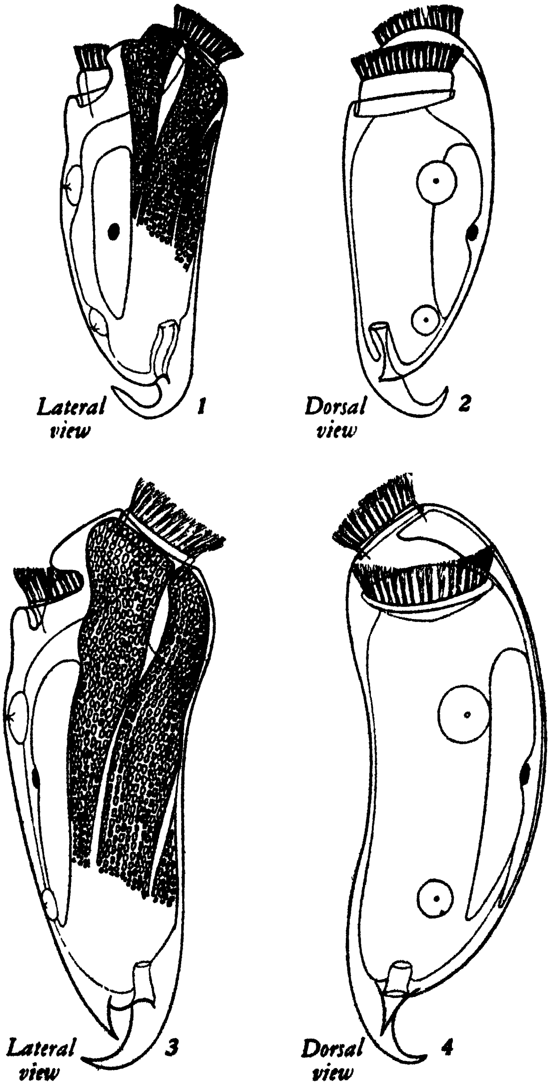


Fig. A. 1 and 2, *Epidinium caudatum* (Fiorentini 1889); 3 and 4, *Epidinium bicaudatum* (Sharp 1914). × 500

Occurrence.—*Epidinium caudatum* is found in two of the *Bos indicus* from Colombo, Ceylon. The most common host of *E. caudatum* is domestic cattle (*Bos taurus*). This has been reported from Italy by Fiorentini (1889); from California by Sharp (1914); from Iowa by Becker and Talbott (1927); from Brazil by Cunha (1914); from the U.S.S.R. by Dogiel (1927), and from Uganda by Dogiel (1932). It has also been reported from reindeer (*Rangifer tarandus*) and deer (*Cervus canadensis ludorfi*) from northern U.S.S.R. by Dogiel (1927); from the camel (*Camelus dromedarius*) from Turkestan by Dogiel (1926); and from the Kaffir buffalo (*Syncerus caffer*) from Uganda by Dogiel (1932).

Relationships.—*E. caudatum* is the second member of the *E. ecaudatum* group and is distinguished mainly by the presence of a single ventral spine.

Epidinium bicaudatum (Sharp 1914)

Plate 1, figure 4; figure A, 3–4

Diplodinium ecaudatum forma bicaudatum Sharp 1914, p. 92, pl. 5, fig. 7.
Epidinium bicaudatum Crawley 1923, p. 412.
Epidinium ecaudatum forma bicaudatum Dogiel 1927, pp. 166–167, figs. 94, 95c.

Diagnosis.—Body elongate (2.10–2.88 dorso-ventral diameters in length) and tapered posteriorly; a large ventral spine, a smaller dorsal spine. Length 82–144μ.

Description.—The body is elongate (2.10–2.88 dorso-ventral diameters in length) and tapering toward the posterior end. The ventral and left surfaces are concave, the dorsal and right surfaces strongly convex. The oral area (0.33–0.52 dorso-ventral diameter in diameter) is inclined ventrally at an angle of 30–50° and to the left at an angle of 30–45°.

The large ventral caudal spine arises from the postero-ventral end of the body at the left of the mid-line. It curves dorsally and to the right. It is homologous in position and shape to the spine of *E. caudatum*. The small dorsal spine arises from the postero-dorsal end of the body on the mid-line. It curves ventrally.

The cuticle is covered with fine longitudinal striations. Beneath the cuticle, three skeletal plates extend from the operculum and the right side of the oral area posteriorly past the middle of the body. The ectoplasm is relatively thin except in the anterior end of the body in the region of the locomotor organelles.

The macronucleus is an elongate body (1.00–1.86 dorso-ventral diameters in length) lying beneath the right dorsal surface adjacent to the edge of the dorsal skeletal plate. It is somewhat narrower and longer than the macronucleus of *E. caudatum*. The micronucleus is a small ellipsoidal body 5–8μ in diameter. It lies in a shallow depression in the right dorsal side of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface, slightly to the right of the mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly and to the right from the mouth. The longitudinal fibrils lining it are fine. The boundary layer is well developed. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a narrow ectoplasmic structure in the postero-ventral end of the body. It is lined with fine, longitudinal fibrils. The anus is a narrow, elliptical opening near the right dorsal side of the base of the ventral spine.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus.*

Axis	Microns	Proportional
Length	118(82–144)	2.37(2.10–2.88)
Transdiameter	46(38– 55)	0.92(0.83–1.00)
Dorso-ventral diameter	50(39– 60)	1.00
Macronucleus	67(38– 93)	1.35(1.00–1.86)
Oral area	22(19– 29)	0.44(0.33–0.52)
Caudal spines	26(20– 30)	0.51(0.33–0.65)

Occurrence.—*E. bicaudatum* is present in small numbers from material from one *Bos indicus* collected in Colombo, Ceylon. Sharp (1914) found it in cattle from California, and Dogiel (1927) in cattle in the U.S.S.R.

Relationships.—*E. bicaudatum* is the third member of the *E. ecaudatum* group.

Epidinium tricaudatum (Sharp 1914)

Plate 1, figure 2; figure B, 1–2

Diplodinium ecaudatum forma *tricaudatum* Sharp 1914, p. 92, pl. 5, fig. 8; Buisson 1923*a*, p. 117, fig. 29; Becker and Talbott 1927, p. 355, fig. 25*a*.

Epidinium tricaudatum Crawley 1923, p. 412.

Ophryoscolex ecaudatus forma *tricaudatus* Dogiel 1926, p. 253.

Epidinium ecaudatum forma *tricaudatum* Dogiel 1927, pp. 167–168, fig. 95*a–b*.

Diagnosis.—Body elongate (2.02–2.50 dorso-ventral diameters in length) and tapered posteriorly; a large ventral spine, a dorsal spine and a right lateral spine. Length 85–131μ (10 specimens).

Description.—The body is elongate (2.02–2.50 dorso-ventral diameters in length) and is tapered toward the posterior end. The ventral and left surfaces are flat or slightly concave, the dorsal and right surfaces convex. The oral area (0.37–0.52 dorso-ventral diameter in diameter) is inclined ventrically at an angle of 30–45° and to the left at an angle of 35–40°.

The ventral spine, the largest of the three, arises from the postero-ventral end of the body at the left of the mid-line. It curves dorsally and to the right. There is a small dorsal spine homologous to the dorsal spine of *E. bicaudatum.* A small lateral spine arises from the right side near the base of the ventral spine. The dorsal and lateral spine vary from small points (fig. B, 1–2) to large spikes only slightly smaller than the ventral spine.

The cuticle is covered with fine longitudinal striations. Beneath the cuticle of the right and ventral sides are the three skeletal plates. They extend from the edge of the oral area posteriorly to the level of the posterior end of the macronucleus. The ectoplasm is relatively thin except in the anterior end of the body in the region of the locomotor organelles.

The macronucleus is an elongate body 0.47–1.52 dorso-ventral diameters in length. It lies beneath the right dorsal surface adjacent to the edge of the dorsal skeletal plate. The micronucleus is a small ellipsoidal body, 3–7μ in diameter, lying in a shallow depression in the middle of the right dorsal side of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface, at the right of the mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly and to the right from the mouth. Fine longitudinal fibrils line it. The boundary layer is conspicuous. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a narrow ectoplasmic structure in the postero-ventral end of the body. Fine longitudinal fibrils may be seen in its walls. The narrow elliptical anus lies between the bases of the ventral and the lateral spines.

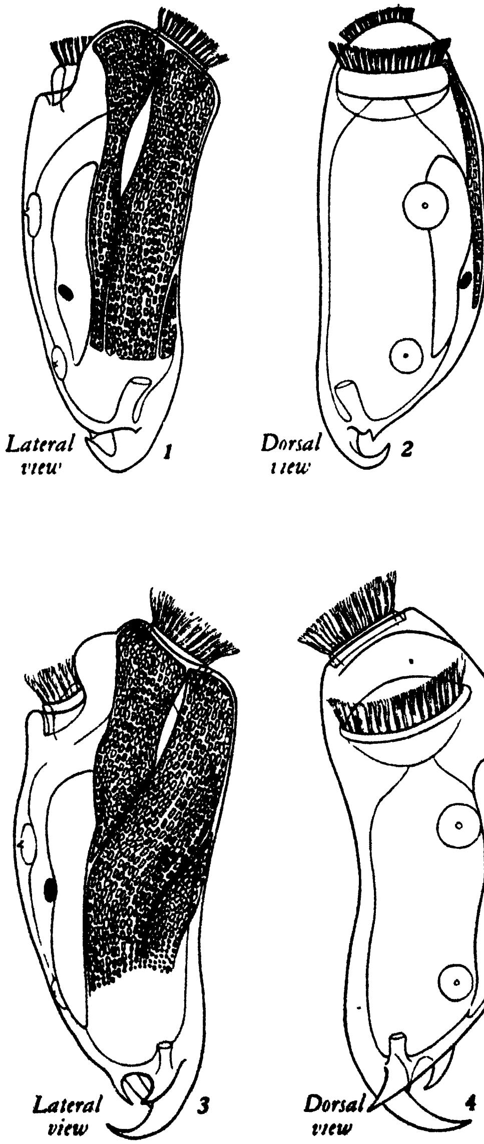


Fig. B. 1 and 2, *Epidinium tricaudatum* (Sharp 1914); 3 and 4, *Epidinium quadricaudatum* (Sharp 1914). ×500

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from ten specimens picked at random from *Bos indicus.*

Axis	Microns	Proportional
Length	112(85–131)	2.31(2.02–2.50)
Transdiameter	46(40– 58)	0.94(0.85–1.00)
Dorso-ventral diameter	49(42– 60)	1.00
Macronucleus	60(19– 80)	1.23(0.47–1.52)
Mouth	21(19– 22)	0.44(0.37–0.52)
Caudal Spines	24(5– 35)	0.50(0.12–0.75)

Occurrence.—*E. tricaudatum* is present in small numbers in material from one *Bos indicus* from Colombo, Ceylon. Sharp (1914) reported it from domestic cattle from California, and Dogiel (1927) from the U.S.S.R. It has also been reported from the Kaffir buffalo (*Syncerus caffer*) from Uganda by Dogiel (1932).

Relationships. E. tricaudatum is the fourth member of the *E. ecaudatum* group.

Epidinium quadricaudatum (Sharp 1914)

Plate 1, figure 3; figure B, 3–4

Diplodinium ecaudatum forma *quadricaudatum* Sharp 1914, pp. 93–94, pl. 5, fig. 9; Buisson 1923*a*, p. 117, fig. 39.

Epidinium quadricaudatum Crawley 1923, p. 412.

Ophryoscolex ecaudatus forma *quadricaudatus* Dogiel 1926, p. 254.

Epidinium ecaudatum forma *quadricaudatum* Dogiel 1927, pp. 168–169, fig. 96*a–b*.

Diagnosis.—Body elongate (2.27–2.32 dorso-ventral diameters in length) and tapered posteriorly; a large ventral spine, a dorsal spine, and two right lateral spines. Length 110–119μ (10 specimens).

Description.—The body is elongate (2.27–2.32 dorso-ventral diameters in length) and is tapered toward the posterior end. The ventral and left surfaces are strongly concave, the dorsal and right surfaces convex. The oral area (0.38–0.48 dorso-ventral diameter in diameter) is inclined ventrally at an angle of about 40° and to the left at an angle of about 35°.

The large ventral spine (0.38–0.69 dorso-ventral diameter in length) arises from the postero-ventral end of the body at the left of the mid-line. It curves dorsally and to the right. There is a small dorsal spine homologous to the dorsal spine in *E. bicaudatum* and *E. tricaudatum.* There are two right lateral spines in place of the single lateral spine of *E. tricaudatum.* The ventral spine is always the longest, the two lateral spines are the shortest and nearly equal in length, and the dorsal spine is slightly larger than the lateral spines.

Fine striations extend longitudinally over the cuticle. The three skeletal plates lie beneath the right and ventral sides. They extend from the edge of the oral area posteriorly to the level of the posterior end of the macronucleus. The ectoplasm is relatively thin except in the anterior end of the body.

The elongate macronucleus (1.29–1.42 dorso-ventral diameters in length) lies beneath the right dorsal surface adjacent to the edge of the dorsal skeletal plate. The small ellipsoidal micronucleus, 5–6μ in diameter, lies in a shallow depression in the middle of the left dorsal side of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface at the right of the mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly and to the right from the mouth. It is lined with fine longitudinal fibrils. The boundary layer, separating ectoplasm and endoplasm, is clearly marked. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a narrow, ectoplasmic structure in the postero-ventral end of the body. Fine longitudinal fibrils may be seen in its walls. The anus lies between the bases of the ventral and lateral spines.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from ten specimens picked at random from *Bos indicus.*

Axis	Microns	Proportional
Length	116(110–119)	2.29(2.27–2.32)
Transdiameter	47(40– 50)	0.92(0.83–0.98)
Dorso-ventral diameter	51(48– 52)	1.00
Macronucleus	69(62– 74)	1.38(1.29–1.42)
Oral area	22(20– 23)	0.43(0.38–0.48)
Caudal spines	26(20– 35)	0.52(0.38–0.69)

Occurrence.—*E. quadricaudatum* is present in small numbers in material from one *Bos indicus* from Colombo, Ceylon. Sharp (1914) reported it from domestic cattle from California, and Dogiel (1927) from the U.S.S.R.

Relationships.—*E. quadricaudatum* is the fifth member of the *E. ecaudatum* group.

Epidinium parvicaudatum (Awerinzew and Mutafowa 1914)

Diplodinium ecaudatum forma *cattanei* Sharp 1914, pp. 94–95, pl. 3, figs. 4, 5; Becker and Talbott 1927, p. 355.

Ophryoscolex fasciculus forma *parvicaudata* Awerinzew and Mutafowa 1914, p. 114, pl. 9, fig. 5.

Epidinium ecaudatum forma *cattaneoi* Dogiel 1927, pp. 169–171, fig. 97; 1932, p. 97.

Diagnosis.—Body relatively long (2.4–2.8 dorso-ventral diameters in length) and tapered posteriorly; five short incurved caudal spines, one rising from the ventral side, two from the dorsal side, and one from each lateral surface. Length, 82–160μ.

Occurrence.—*E. parvicaudatum* was reported by Awerinzew and Mutafowa (1914) from cattle from Leningrad; by Sharp (1914 from cattle from the western U. S.; and by Dogiel (1927, 1932) from various parts of the U.S.S.R. and from Uganda.

Relationship.—*E. parvicaudatum* has the greatest complexity of spination of all species of the *E. ecaudatum* group. The size and shape of the spines and the tapering of the body clearly place it with this group and separate it from the long-spined *E. cattanei.*

Sharp (1914) identified his five-spined species as *Diplodinium cattanei* Fiorentini (1889), but at the same time pointed out serious discrepancies between his description and that of Fiorentini. Other authors (Awerinzew and Mutafowa 1914; Dogiel 1927), with the opportunity to study both types, have separated them. Awerinzew and Mutafowa gave the short-spined type the forma name *parvicaudata.* The name *E. cattanei* (Fiorentini 1889) clearly belongs to the long-spined species.

HAMATUM GROUP

This group is characterized by the heavy posterior half of the body, not tapered as in the *Epidinium ecaudatum* group, but nearly as stout as the anterior half of the body. The posterior end is extended to form a large dorsal lobe. The endoplasmic sack extends posteriorly into this lobe beyond the level of the rectum. *E. hamatum* possesses a narrow ventral spine, while *E. bulbiferum* has a peculiar bulb-like ventral projection.

Epidinium hamatum (Schulze 1924)

Diplodinium hamatum Schulze 1924, p. 656, figs. 2–4, 6–11; Dogiel 1926, pp. 248, 267.
Ophryoscolex ecaudatus forma *hamatus* Dogiel 1926, pp. 248–250, fig. 3.
Epidinium ecaudatum forma *hamatum* Dogiel 1927, pp. 163–164, fig. 92.

Diagnosis.—Body elongate (2.5 dorso-ventral diameters in length); posterior part of body thick and heavy; dorsal side projects posteriorly beyond level of anus; a thin ventral spine present. Length 104–150μ.

Occurrence.—*E. hamatum* was first reported by Schulze (1924) from cattle from Germany. Dogiel (1927) reports it from cattle and sheep from various parts of the U.S.S.R. and from the camel (*Camelus dromedarius*) from Turkestan.

Relationships.—The heavy, blunt posterior half of *E. hamatum* separates this species from *E. caudatum* with its subconical posterior end.

Epidinium bulbiferum (Dogiel 1927)

Epidinium ecaudatum forma *bulbiferum* Dogiel 1927, pp. 165–166, fig. 93*a–d*.

Diagnosis.—Body elongate (2.6 dorso-ventral diameters in length); posterior part of body thick and heavy; dorsal side projects posteriorly beyond level of anus; rounded ventral lobe with its base narrower than its median diameter. Length 98–150μ.

Occurrence.—*E. bulbiferum* is reported by Dogiel (1927) from domestic cattle (one host only) from the U.S.S.R.

Relationships.—This species is similar to *E. hamatum* in the general form and size of the body, but the ventral spine present in *E. hamatum* is replaced by a lobe in *E. bulbiferum*.

UNALLOCATED SPECIES

Three species of *Epidinium*, *E. gigas*, *E. cattanei*, and *E. eberleini*, do not fit in with the *E. ecaudatum* group or the *E. hamatum* group. *E. gigas* is separated from the other species of the genus by its size, untapered posterior end, and by differences in the skeletal complex; *E. cattanei* by the truncated posterior end and by peculiarly shaped and very long spines; *E. eberleini* by the presence of an accessory skeletal plate in the main caudal spine, and by the presence of two lateral lobes. No other species of the genus shows either lobes or accessory skeletal plates.

Epidinium gigas (Dogiel 1925)

Ophryoscolex ecaudatus forma *gigas* Dogiel 1925*c*, pp. 58, 59, 61, figs. 19, 20.
Epidinium ecaudatum forma *gigas* Dogiel 1927, pp. 173–175, fig. 99.

Diagnosis.—Body relatively heavy (2.35 dorso-ventral diameters in length) and tapered posteriorly; posterior end smoothly rounded. Length 135–230μ.

Occurrence.—*E. gigas* is reported by Dogiel (1927) from reindeer (*Rangifer tarandus*) from northern U.S.S.R.

Relationships.—*E. gigas* is similar in general form of the body to *E. ecaudatum* and differs from it mainly in size. The left skeletal plate is larger than in *E. ecaudatum* and the posterior half is expanded dorsally. Also, the anterior half of the macronucleus is bent ventrally.

Epidinium cattanei (Fiorentini 1889)

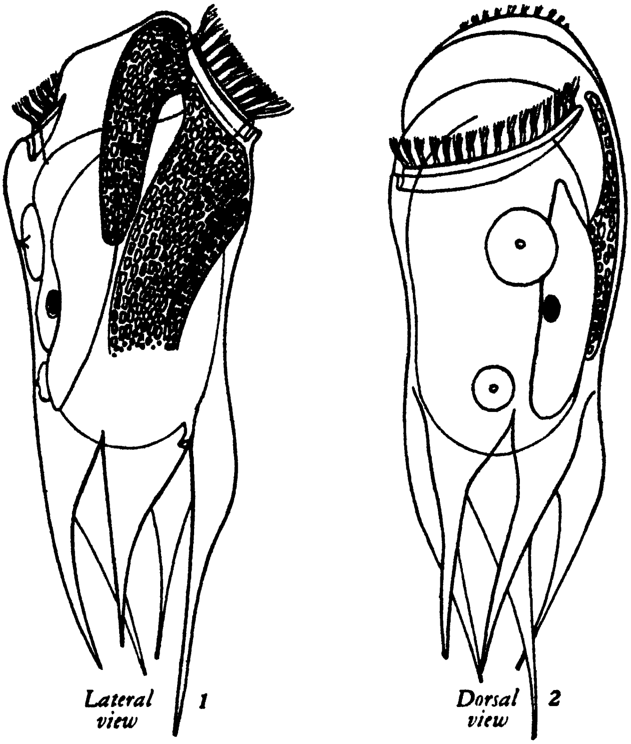
Plate 1, figure 7; figure C, 1–2

Diplodinium cattanei Fiorentini 1889, pp. 16–17, pl. 3, figs. 4–5.
Diplodinium ecaudatum forma *cattanei* Becker and Talbott, partim, 1927, p. 355 (see *Epidinium parvicaudatum*); non Sharp 1914, pp. 94–95, pl. 5, fig. 10 (see *E. parvicaudatum*).
Diplodinium ecaudatum var. *cattanei* Buisson 1923*a*, pp. 118–119, fig. 42.
Ophryoscolex cattanei Railliet 1890.
Ophryoscolex cattaneoi Cunha 1914*b*, pp. 62, 63.
Ophryoscolex fasciculus Awerinzew and Mutafowa, partim, 1914, pp. 112–114, fig. 4 (for p. 114, pl. 9, fig. 5, see *Epidinium parvicaudatum*).
Epidinium cattanei Crawley 1923, p. 412.
Ophryoscolex ecaudatus cattaneoi Dogiel 1926, p. 254.
Epidinium ecaudatum forma *fasciculus* Dogiel 1927, pp. 171–173, fig. 98.
Epidinium caudatum forma *fasciculus* Dogiel 1927, p. 171.
non *Epidinium ecaudatum* forma *cattanei* Dogiel 1927, pp. 169–171, fig. 97 (see *E. parvicaudatum*).

Diagnosis.—Body relatively short (1.63–2.38 dorso-ventral diameters in length) and truncate posteriorly; five long straight caudal spines, one arising from the ventral side, two from the dorsal side, and one from each lateral surface. Length 78–120μ (10 specimens).

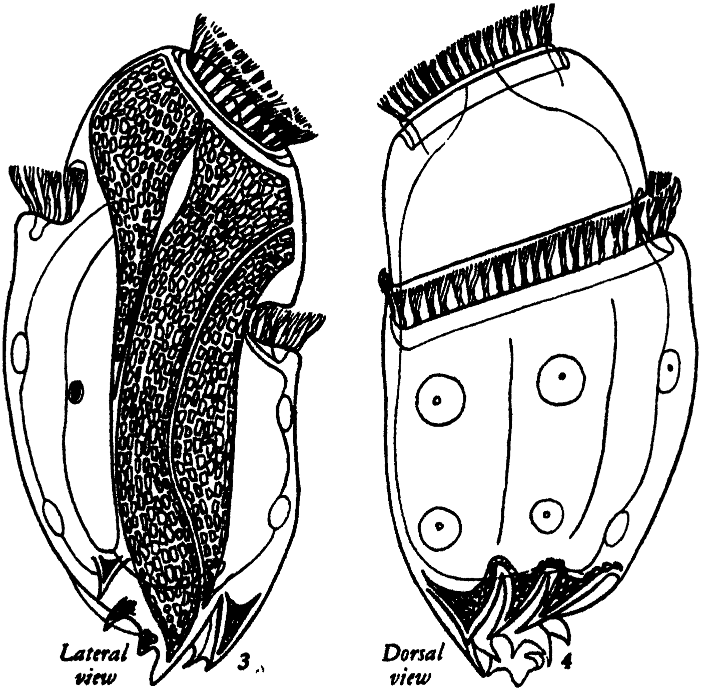
Description.—The body is relatively short and heavy and is truncated posteriorly. This is in sharp contrast to the narrow, posteriorly tapering form shown by *E. caudatum, E. quinquecaudatum,* and the other members of Sharp's group. The ventral surface of *E. cattanei* is slightly concave. The left and dorsal surfaces are concave between the dorsal membranelle zone and the base of the left spine. The right surface is convex. The oral area (0.43–0.67 dorso-ventral diameter in diameter) is inclined ventrally at an angle of about 45° and to the left at an angle of about 35°.

The five caudal spines (0.87–1.41 dorso-ventral diameters in length) are the largest and most prominent spines found in any of the Ophryoscolecidae. The ventral spine is only slightly larger than the other four spines. Each spine arises from a relatively broad base, but tapers rapidly in the proximal third, so that the distal two-thirds are relatively thin. The spines are relatively straight and show little tendency to curve inward, even in fixed material. The spines of *E. cattanei* contrast strongly with the short, uniformly tapering, inward curved spines of *E. quinquecaudatum.* This difference is shown in all



Lateral view 1

Dorsal view 2



Lateral view 3

Dorsal view 4

Fig. C. 1 and 2, *Epidinium cattanei* (Fiorentini 1889);
3 and 4, *Ophryoscolex spinosus* sp. nov.
×500

the published drawings of the two species. The largest spine projects from the ventral side of the posterior end of the body, two spines from the dorsal side, and one from each side between the ventral and dorsal spines.

The cuticle is covered with fine longitudinal striations, as in the other species of *Epidinium*. The anterior two-thirds of the three skeletal plates extend around the oral area from the operculum to the ventral side. They gradually narrow posteriorly and cover less than half of the right side. The dorsal plate is usually shorter than the other two and often ends near the level of the anterior contractile vacuole. The middle and ventral plates terminate posteriorly a short distance behind the middle of the body. All three plates are relatively shorter than the plates of *E. ecaudatum* and the other species closely related to it. The ectoplasm is relatively thin except in the anterior end of the body in the region of the locomotor organelles.

The macronucleus is an elongate body (0.85–1.45 dorso-ventral diameters in length) lying beneath the right dorsal surface adjacent to the edge of the dorsal skeletal plate. The micronucleus is a small ellipsoidal body 4–7μ in diameter. It lies in a shallow depression in the dorsal side of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface. The large anterior vacuole is located at the right of the mid-line, while the smaller posterior vacuole is located very near the mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly from the mouth and towards the right surface. The fibrils lining it are fine. The boundary layer is conspicuous. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a small, narrow, ectoplasmic structure in the postero-ventral end of the body. It is relatively smaller than in *E. ecaudatum*. The anus is a narrow elliptical opening near the dorsal side of the base of the ventral spine.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus*.

Axis	Microns	Proportional
Length	97 (78–120)	1.84 (1.63–2.38)
Transdiameter	50 (40– 61)	0.94 (0.87–0.98)
Dorso-ventral diameter	53 (42– 65)	1.00
Macronucleus	55 (40– 71)	1.04 (0.85–1.45)
Oral area	27 (22– 32)	0.53 (0.43–0.67)
Caudal spines	63 (4– 80)	1.17 (0.87–1.41)

Occurrence.—*Epidinium cattanei* is present in small numbers in material from one *Bos indicus* from Coonoor, India, and from two *Bos indicus* from Colombo, Ceylon. *E. cattanei* has been reported from domestic cattle (*Bos taurus*) from Italy by Fiorentini (1889); from the U.S.S.R. by Awerinzew and Mutafowa (1914) and Dogiel (1927); from France by Railliet (1890) and Buisson (1923); from Iowa by Becker and Talbott (1927); from Brazil by Cunha (1914).

Relationships.—The relatively short truncated body of *E. cattanei* and the peculiar shape of the very long spines separate it from the other species of the genus.

Epidinium eberleini (Cunha 1914)

Plate 1, figure 5; figure D

Diplodinium caudatum Eberlein 1895, pp. 260–261, fig. 16.
Diplodinium eberleini Cunha 1914*b*, p. 62; Sharp 1914, pp. 51, 61; Buisson 1923*a*, pp. 118–120, fig. 36; Dogiel 1927, p. 156, fig. 89; Becker and Talbott 1927, p. 355.
Diplodinium longispinum Schulze 1924, p. 656.
Epidinium lobatum Dogiel 1928, pp. 334–337, fig. 5*a*–*b*.

Diagnosis.—Body elongate (1.82–2.32 dorso-ventral diameters in length); broad, laterally flattened caudal lobe on right side; a blunt caudal lobe on left side; a long ventral spine; an accessory skeletal plàte in ventral spine. Length 85–118μ (10 specimens).

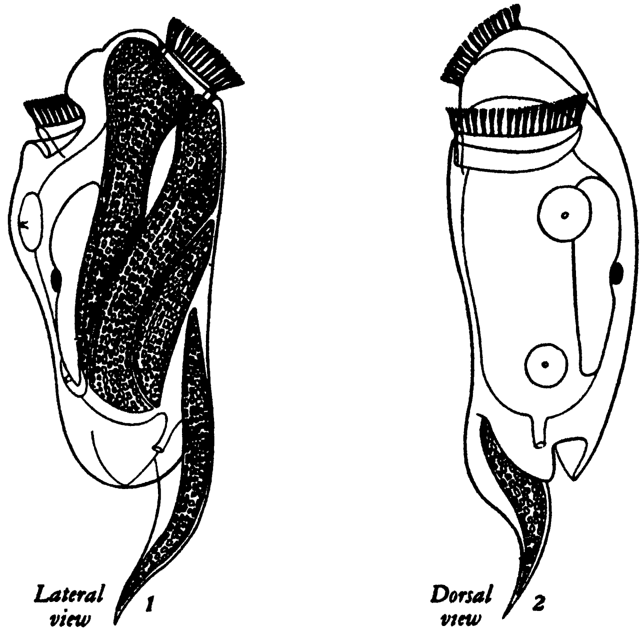


Fig. D. 1 and 2, *Epidinium eberleini* (Cunha 1914). ×500

Description.—The body is elongate (2.04–2.86 dorso-ventral diameters in length) with a relatively blunt posterior end. The ventral and left surfaces are nearly plane, the right surface is strongly convex. The anterior half of the dorsal surface is convex, the posterior half concave or plane. The oral area (0.34–0.52 dorso-ventral diameter in diameter) is inclined ventrally and to the left at an angle of about 45°.

The large caudal spine (0.75–1.40 dorsoventral diameters in length) arises on the left ventral side of the posterior end. In dorsal view it has the form of a sigmoid curve (fig. D, 2). The right side of the body is continued posteriorly as a broad, laterally flattened lobe. A narrower, heavier lobe projects from the posteior end of the left surface.

The cuticle is covered with fine longitudinal striations. Beneath the cuticle three skeletal plates extend from the operculum and the right side of the oral area to the level of the posterior end of the macronucleus. These form the main skeletal complex. A long, thin accessory skeletal plate extends from the middle of the ventral surface to the tip of the large ventral spine. The ectoplasm is relatively thin except in the anterior region of the body.

The macronucleus is an elongate body (1.00–1.37 dorsoventral diameters in length) adjacent to the dorsal edge of the main skeletal complex. The micronucleus is a small, ellipsoidal body 5–8μ in diameter lying in a shallow depression in the middle of the right dorsal surface of the macronucleus.

The two contractile vacuoles lie beneath the dorsal surface. The anterior vacuole is at the right of the mid-line, while the posterior vacuole is on the mid-line. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly from the mouth, the fibrils continuing along the right side to the posterior end of the body. The boundary layer is well developed. The endoplasmic sack extends from the level of the dorsal membranelle zone to the posterior end of the body. The rectum is a narrow, ectoplasmic structure in the postero-ventral end of the body. The anus opens at the base of the ventral spine between the two lateral lobes.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from ten individuals picked at random from *Bos indicus.*

Axis	Microns	Proportional
Length	103 (85–118)	2.10 (1.82–2.32)
Transdiameter	45 (39– 60)	0.92 (0.85–0.98)
Dorso-ventral diameter	49 (42– 65)	1.00
Macronucleus	56 (40– 80)	1.14 (1.00–1.37)
Oral area	21 (20– 23)	0.44 (0.34–0.52)
Caudal spines	50 (30– 70)	1.04 (0.75–1.40)

The measurements given by Eberlein are as follows: length 100–120μ; dorso-ventral diameter 60–70μ. Dogiel (1928) gives the following measurements: length 72–105μ; dorso-ventral diameter 37–58μ; caudal spine 40–60μ.

Occurrence.—*Epidinium eberleini* is present in two of the *Bos indicus* from Coonoor, India, and in two from Colombo, Ceylon. It was reported in domestic cattle from Germany by Eberlein (1895), and Schulze (1924); from *Buffelus bubalus* from Turkestan by Dogiel (1928).

Relationships.—*E. eberleini* is separated from the other species of the genus by the unique appearance of its caudal lobes and by the presence of the accessory skeletal plate in the ventral spine.

EPIPLASTRON gen. nov.

Epidinium Dogiel 1928, *partim*, pp. 181–183, figs. 100–102 (for pp. 156–181, figs. 90–99, see *Epidinium*).

Diagnosis.—Body elongate; dorsal membranelle zone behind the anterior end of the body; main skeletal complex composed of five plates; macronucleus a straight elongate body; two contractile vacuoles.

Type species.—*Epiplastron africanum* (Dogiel 1925) from antelope (*Rhaphiceros* sp.) from British East Africa.

CHARACTERS OF SYSTEMATIC IMPORTANCE

Dogiel (1927) has given a thorough description of the skeletal plates of *Epiplastron,* but has given very little information on the rest of its morphology, merely stating that it is similar to *Epidinium ecaudatum.*

The skeletal complex of five plates is located similarly to that of *Epidinium* and *Ophryoscolex.* The anterior ends of the plates surround the oral area except on the left side and terminate posteriorly on the right side near the level of the posterior end of the macronucleus. The skeletal plates are most conveniently designated by numbers running dorso-ventrally—the dorsal plate is number one, the ventral plate number five. Plate one arises on the dorsal edge of the oral area and extends posteriorly and to the right, terminating near the anterior end of the macronucleus. Plates two, three, and four arise on the right side of the oral area and extend posteriorly to the level of the posterior end of the macronucleus. These plates are fused together in the posterior half of the body. Plate five arises on the ventral edge of the oral area and extends posteriorly and to the right. It extends approximately the same distance posteriorly as plate one. The posterior end of plate five may be fused with plate four. In the anterior half of the body the plates are separated from each other by narrow areas of undifferentiated ectoplasm. The dorsal membranelle zone lies slightly behind the anterior end of the body and is of the same size as in *Epidinium.* There are two contractile vacuoles lying beneath the dorsal surface. The macronucleus is a straight, elongate body lying beneath the right dorsal surface, adjacent to the dorsal edge of the skeletal complex.

Epiplastron appears to be an offshoot from *Epidinium* along the line of increase in the number of main skeletal plates from three to five, and an increase in size of the whole skeletal complex.

The recent description of *Epiplastron spinosum* by Dogiel (1932) shows in this genus the beginning of a series of species showing progressive caudal spination similar to that in the *Epidinium ecaudatum* series.

Epiplastron africanum (Dogiel 1925)

Figure E

Ophryoscolex ecaudatus forma *africanus* Dogiel 1925*b*, p. 136, fig. 7.
Epidinium africanum Dogiel 1927, pp. 181–183, figs. 100–102.

Diagnosis.—Body relatively long (2.55 dorso-ventral diameters in length) and tapered posteriorly; posterior end smoothly rounded. Length 90–140μ.

Occurrence.—*Epiplastron africanum* was reported by Dogiel (1925) from the antelope (*Rhaphiceros* sp.) from British East Africa.

Epiplastron spinosum (Dogiel 1932)

Epidinium africanum forma *spinosum* Dogiel 1932, p. 104.

Diagnosis.—Body relatively long (2.3 dorso-ventral diameters in length) and tapered posteriorly; single, long, narrow ventral spine. Length 98–112μ.

Occurrence.—*Epiplastron africanum* was reported from the impala antelope (*Aepyceros melampus*) from Africa.

Relationships.—Dogiel (1932) gave a very short description and no figures of this species. He states, however, that it differs from *Epiplastron africanum* only in the presence of the long ventral spine.

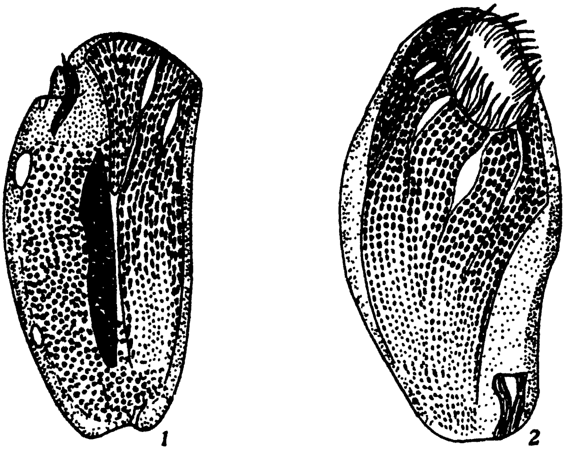


Fig. E. *Epiplastron africanum* (Dogiel 1925). 1, right lateral view; 2, ventral view. Redrawn after Dogiel 1927. x 500

OPHRYOSCOLEX Stein

Ophryoscolex Stein 1858, p. 69; 1859, pp. 57–58; Eberlein 1895, pp. 239–251, pl. 16, figs. 1–7; Cunha 1914*d*, *partim*, p. 6 (see *Epidinium*); 1914*b* non *Ophryoscolex* (for pp. 60–63, pl. 7, fig. 1, see *Epidinium*); Buisson 1923*b*, p. 237; Dogiel 1923*b*, non *Ophryoscolex* (for pp. 219–232, pl. 17, see *Opisthotrichum*); 1925*b*, *partim*, pp. 134–135 (for pp. 136–137, fig. 7, see *Epidinium*); 1925*c* non *Ophryoscolex* (for pp. 57–58, pl. 2, figs. 18–20, see *Epidinium*); 1927, pp. 183–211, figs. 103–117; Becker and Talbott 1927, pp. 358–359, pl. 3, figs. 26–27.

Diplodinium Fiorentini 1889, *partim*, pp. 11–12, pl. 1, figs. 1–2 (for pp. 13–14, pl. 1, figs. 3–4, pl. 2, figs. 1–2, see *Eudiplodinium*; for p. 14, pl. 2, fig. 3, see *Ostracodinium*; for p. 15, pl. 2, figs. 4–5, see *Diplodinium*; for pp. 15–16, 17, pl. 3, figs. 1–2, 4–5, see *Epidinium*; for p. 16, pl. 3, fig. 3, see *Eremoplastron*).

Diagnosis.—*Ophryoscolecidae* with dorsal membranelle zone forming a girdle extending three-fourths the distance around the middle of the body and open only on right ventral side; skeletal complex formed of three plates extending the length of the right ventral side; 9–15 vacuoles ranged around body in two transverse rows.

Type species.—*Ophryoscolex inermis* Stein 1858 from domestic cattle from the C.S.R. No type species was designated either by Stein or by later workers until Buisson (1923*b*) made a revision of the Ophryoscolecidae based on previous literature. He unfortunately selected as type species *O. inermis,* which has been figured only by Eberlein (1895), whose description and figure are manifestly inadequate, particularly in regard to the macronucleus, number and position of the vacuoles, presence of skeletal plates, etc. It has been reported since then only by Cunha (1914) and by Becker and Talbott (1927), and they do no more than indicate its presence. Thus the most inadequately known and rarest species of the genus is the type species!

REVIEW OF LITERATURE

Ophryoscolex was first described by Stein (1858) as the type genus of the family he erected at the same time. He gave a very good description including the oral region and its method of retraction, the median girdle of cilia, ectoplasm, endoplasm, anus, nuclei, and contractile vacuoles. He described *O. purkynjei,* a common species with a complex array of caudal spines, and *O. inermis,* a rare species with a smooth posterior end. He found them to be most abundant in sheep, and present in cattle only in small numbers. Fiorentini (1889) redescribed *O. purkynjei* as *Diplodinium vortex.* Eberlein (1895) described *O. caudatus* and gave the first thorough description of its morphology. Günther (1897) added an account of division. Braune (1914) described parts of the complex fibrillar system. Dogiel (1927) described four new forms (which we consider as species) and in addition did considerable work on the variation of spination in this group. One new species is reported in this paper from *Bos indicus,* bringing the total number of species of *Ophryoscolex* to nine.

Ophryoscolex has to some extent reflected the early uncertainty in regard to the proper generic limits of *Diplodinium.* Fiorentini (1889) placed *Ophryoscolex purkynjei* in *Diplodinium.* Later Cunha (1914), Awerinzew and Mutafowa (1914), Dogiel (1925) placed the *D. ecaudatum* group in *Ophryoscolex.* Dogiel (1923) placed a species from African antelope in this genus, but Buisson (1923*b*) used it as the type of a new genus *Opisthotrichum.* Crawley (1923) placed the *D. ecaudatum group* in a separate genus, *Epidinium,* and since then *Ophryoscolex* has been the most clearly defined and most stable genus of the family.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The size and position of the membranelle zones are the most obvious and most important characteristics of this genus. The adoral zone is relatively small (about one-third the diameter of the body) and inclined ventrally and to the left at an angle of about 45°. The row of membranelles homologous to the dorsal membranelle zone in *Epidinium* and other genera is elongated and shifted posteriorly, so that it forms a median girdle around three-quarters of the circumference of the body. The right end of the girdle lies just dorsal to the skeletal complex in the anterior third of the body, spirals to the left and slightly posteriorly, and terminates just ventral to the skeletal complex in the middle of the body. The great increase in the length of the dorsal membranelle zone as compared with the homologous zone in *Epidinium* is accomplished entirely through an extension of the left side of the zone. The position of the dorsal zone in the middle of the body has changed the operculum from a small region, such as occurs in *Epidinium, Polyplastron,* etc., into a region comprising approximately one-third of the whole body.

The main skeletal complex is composed of three long skeletal plates lying beneath the right ventral side of the body. The three plates are shaped anteriorly just as the plates in *Epidinium,* and partly surround and support the oral region. The dorsal plate terminates in the middle of the body as in *Epidinium.* The right and the ventral plates, however, continue posteriorly to the end of the body and even extend for a distance into the main caudal spine. The secondary spines also contain accessory plates conforming in shape to the shape of the spines. The plates within the spines of the anterior circlet are often connected by short cross-bars to form a single skeletal complex. These secondary skeletal plates are as variable as the spines in which they occur.

Longitudinal grooves extending posteriorly from the dorsal membranelle zone divide the body into six or seven antimeres. Two of these are occupied respectively by the skeletal plates and the macronucleus. There is usually a pair of vacuoles in each of the other antimeres.

A complex array of caudal spines is characteristic of all species of *Ophryoscolex* (except *O. inermis,* a doubtful species). There is a large main spine, formed by a continuation of the antimere containing the main skeletal complex. A small secondary spine, either simple or furcate, arises from each of the other antimeres, and these form the first circlet of secondary spines. This is present in all species (except *O. inermis*). Behind this circlet, and partly hidden by it, may be found 1–3 additional circlets of secondary spines.

There are three groups of species in the genus *Ophryoscolex* clearly separated on the basis of number of vacuoles, character of the main spine, etc., but they show a parallel increase of spination by the development of additional rows of secondary caudal spines. In other genera, particularly *Diplodinium* and *Epidinium,* the increase in complexity of spination is by the addition of single spines, but in *Ophryoscolex* the increase is accomplished by the addition of complete sets of circlets of secondary spines.

The detailed structure of the caudal complex is one of the most important characters used in the specific classification in this genus. The main spine may be long and slender, or short and stumpy, the secondary spines may be characteristically simple or complexly furcate, and there may be one to four circlets of these. The number of contractile vacuoles varies from 9–15, depending on the species. Other features, such as proportions of the body, the tilt of the oral area, etc., although not so definite as the above characters, are useful as additional characterizations of a species.

Ophryoscolex inermis Stein 1858

Ophryoscolex inermis Stein 1858, p. 70; Eberlein 1895, pp. 240–247, pl. 16, figs. 1–3; Cunha 1914*d*, p. 4; Becker and Talbott 1927, p. 358.

Diagnosis.—Body relatively slender (2.0 dorso-ventral diameters in length); posterior end smoothly rounded. Length 170–190μ.

Occurrence.—*O. inermis* has been reported from goats, sheep, and domestic cattle from the C.S.R. (Stein 1858) and Germany (Eberlein 1895); Brazil (Cunha 1914), and Iowa (Becker and Talbott 1927).

Stein's description of this species merely designates it as a form with a second group of membranelles distinctly separate from the adoral spiral of membranelles and with a smooth posterior end. This description would fit *Epidinium ecaudatum* or *Polyplastron multivesiculatum* equally well. Eberlein's figure at first glance is referable to *Ophryoscolex*, but considering the incorrectness of the drawing of the macronucleus (on the left side, instead of on the dorsal or right side, as in all adequately described species of the family) this drawing also could be referred to *Polyplastron multivesiculatum.* Cunha (1914) and Becker and Talbott (1927) list it, but give no description.

BUISSONI GROUP

The *Ophryoscolex buissoni* group has a long caudal spine similar to that of the *O. caudatus* group and also one to two circlets of secondary caudal spines. There are only five spines in the anterior circlet instead of six as in the *O. caudatus* group and these spines are simple or bifurcate instead of trifurcate as in *O. caudatus.* Thus in spination, the *O. buissoni* group is simpler than the *O. caudatus* group.

There are fifteen vacuoles present in the *O. buissoni* group, ten in the anterior circlet and five in the posterior circlet. In number of vacuoles alone the *O. buissoni* group shows an increase in complexity over the *O. caudatus* group.

There are two species in this group, *O. buissoni* with a single circlet of secondary caudal spines, and *O. bicinctus* with two.

Ophryoscolex buissoni Dogiel 1927

Ophryoscolex buissoni unicinctus Dogiel 1927, pp. 185–192, figs. 103–105.

Ophryoscolex buissoni unicinctus aberration *simpliciispinosus* Dogiel 1927, p. 187, fig. 105.

Diagnosis.—Body relatively stout (2.0 dorso-ventral diameters in length; long, slender main caudal spine; a single circlet of five secondary caudal spines; ten contractile vacuoles in anterior row, five in posterior row. Length 125–165μ.

Occurrence.—Dogiel (1927) reports *O. buissoni* from sheep and cattle (one host) from Sebastopol, U.S.S.R.

Relationships.—This is the simplest form of the genus *Ophryoscolex.*

Ophryoscolex bicinctus Dogiel 1927

Ophryoscolex buissoni forma bicinctus Dogiel 1927, pp. 192–194, figs. 106–107.

Diagnosis.—Body relatively stout (1.8 dorso-ventral diameters in length); long, slender main caudal spine; two circlets of secondary spines, anterior circlet composed of five spines; ten contractile vacuoles in anterior row, five in posterior row. Length 130–170μ.

Occurence.—Dogiel (1927) reports *O. bicinctus* from sheep from Turkestan.

Relationships.—*O. bicinctus* differs from *O. buissoni* only in the addition of a small circlet of spines posterior to the primary circlet.

PURKYNJEI GROUP

The main caudal spine of this group is very short and stubby and may be hardly more than a bluntly pointed lobe. The middle plate of the main skeletal complex extends to the tip of this main caudal spine. There are two to three rows of secondary caudal spines, the anterior row being composed of six spines. There are nine or ten contractile vacuoles arranged in two rows. The number of secondary spines and the number of vacuoles show that this group is more closely allied to the *O. caudatum* group than to the *O. buissoni* group. It is separated from both by the short, heavy main caudal spine.

There are two species in this group, *O. spinosus* with two circlets of secondary caudal spines, and *O. purkynjei* with three circlets.

Ophryoscolex spinosus sp. nov.

Plate 1, figure 6; figure C, 3–4

Diagnosis.—Body relatively slender (1.59–2.14 dorso-ventral diameters in length); main caudal spine short; two rows of accessory caudal spines; spines of anterior row usually simple; ten contractile vacuoles. Length 122–160μ (10 specimens).

Description.—The body is relatively slender (1.59–2.19 dorso-ventral diameters in diameter) compared to the *O. caudatus* and *O. buissoni* groups. The proportions of *O. spinosus* and *O. purkynjei* are very similar. The surfaces of *O. spinosus* are strongly convex except the middle of the ventral side, which is slightly concave, and the anterior two-thirds of the left ventral side, which is nearly plane. The body is divided into seven sectors by shallow, longitudinal grooves. These sectors are particularly well marked posterior to the dorsal membranelle zone. Two of the sectors may be designated by the organelles included within them, i.e., the skeletal sector and the macronuclear sector. It is often convenient to orient other organelles by reference to these main sectors.

The oral area (0.40–0.56 dorso-ventral diameter in diameter) is inclined ventrally at an angle of about 40° and to the left at an angle of about 20°. The anterior end of the dorsal zone arises near the dorsal skeletal plate at the level of the anterior end of the macronucleus. The zone extends to the left and posteriorly, terminating near the ventral skeletal plate at the level of the micronucleus.

The caudal complex is relatively simple as compared with the complex in such forms as *O. caudatus* and *O. purkynjei*. There are two caudal circlets of spines (fig. C, 4). The anterior circlet is composed of simple, or occasionally bifurcate, spines, one for each sector of the body except the skeletal sector. The posterior circlet is just behind the main circlet and partly hidden by it. The spines of the posterior circlet are bifurcate or trifurcate. The number of spines in this circlet is always less than the number of spines of the anterior circlet, usually four or five. The anterior row of spines is stable in number and form of spines, while the posterior row is highly variable. In the skeletal sector the body is prolonged to form the short, heavy, main spine (0.14–0.28 dorso-ventral diameter in length). Two small accessory spines arise on the dorsal side of the main spine, and one on the ventral side. The whole caudal complex of *O. spinosus* is compactly arranged with the spines curved inward. This gives the posterior end a relatively smooth outline continuous with the curvatures of the rest of the body.

The cuticle is covered with fine longitudinal striations. The main skeletal complex is composed of three adjacent plates as in *Epidinium*. The anterior end of the skeletal complex surrounds the oral area except on the left dorsal side. The dorsal plate extends from the operculum to the level of the micronucleus. The median plate extends from the right side of the oral area to the tip of the main caudal spine. The ventral plate extends from the ventral side of the oral area beneath the ventral side of the right surface and terminates near the base of the main caudal spine.

Small triangular accessory skeletal plates occur in most of the caudal spines. The accessory plates of the anterior caudal circlet of spines are usually united to form a single complex by narrow connecting bars between the bases of the spines. The small plates occurring in the other spines do not appear to be connected.

The macronucleus is an elongate body (0.76–1.10 dorso-ventral diameters in length) adjacent to the dorsal edge of the main skeletal complex. The micronucleus is a small ellipsoidal body, 5–8μ in diameter, lying in a shallow depression in the right dorsal side of the macronucleus.

There are ten contractile vacuoles present, arranged in pairs in each sector of the body except the skeletal and macronuclear sectors. The anterior vacuole of each pair is usually the larger. The excretory canals and pores are relatively conspicuous.

The oesophagus extends posteriorly from the mouth and toward the right surface. The fibrils lining it are fine and difficult to see except in sections. The boundary layer is well developed. The endoplasmic sack extends from a level just behind the oral area to the posterior end of the body. The rectum is a narrow, ectoplasmic structure in the postero-ventral end of the body. The anus lies within the anterior circlet of spines near the base of the main caudal spine.

The food consists of bacteria and small flagellates.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus*.

Axis	Microns	Proportional
Length	146 (122–160)	1.92 (1.59–2.14)
Transdiameter	73 (63– 80)	0.97 (0.87–1.00)
Dorso-ventral diameter	75 (63– 82)	1.00
Macronucleus	73 (60– 86)	0.96 (0.76–1.10)
Oral area	36 (30– 40)	0.44 (0.40–0.56)
Caudal Spine	14 (10– 20)	0.19 (0.14–0.28)

Occurrence.—*O. spinosus* is present in small numbers in two of the *Bos indicus* from Coonoor, India.

Relationships.—*O. spinosus* is a type similar to *O. purkynjei,* but with two rows of spines instead of three as in the latter species. These two species are in the same relationship as *O. bicoronatus* and *O. caudatus.*

Ophryoscolex purkynjei Stein 1858

Ophryoscolex purkynjei Stein 1858, p. 70; Eberlein 1895, pp. 250–251, pl. 16, fig. 5; Braune 1913, pp. 43–56; Cunha 1914*b*, pp. 58, 60, 63; Buisson 1923*a*, pp. 128–129, fig. 47; Dogiel and Fedorowa 1926, pp. 257–268, 2 figs; Fantham 1926, p. 568; Dogiel 1927, pp. 206–210, figs. 115–116.

Diplodinium vortex Fiorentini 1889, pp. 11–12, pl. 1, figs. 1–2.

Diagnosis.—Body relatively slender (1.94 dorso-ventral diameters in length); main spine short and stout, with three small spines projecting from it; three circlets of secondary spines; spines of anterior row usually three-pronged; nine contractile vacuoles. Length, 155–215μ.

Occurrence.—*Ophryoscolex purkynjei* has been reported from cattle, sheep, and goats from the C.S.R. by Stein (1858), from Germany by Eberlein (1895) and Braune (1913), from various parts of the U.S.S.R. by Dogiel (1927), from South Africa by Fantham (1926), and from Brazil by Cunha (1914).

Relationships.—The lack of a long ventral spine separates *O. purkynjei* and *O. spinosus,* its most nearly related form, from the other species in the genus.

CAUDATUS GROUP

This group is most clearly characterized by a long, slender main caudal spine. In addition to this main spine there are two to four circlets of secondary spines around the posterior end. The anterior circlet in the species of this group is composed of six spines, each of them usually trifurcate. There are nine contractile vacuoles arranged in two transverse bands around the body, one band just posterior to the median girdle of membranelles (dorsal zone) and the other at the base of the anterior circlet of secondary caudal spines. There are four vacuoles in the anterior row of vacuoles and five in the posterior row. The number of vacuoles and the number of spines of the anterior circlet separate the *Ophryoscolex caudatus* group from the *O. buissoni* group.

There are three species in the *O. caudatus group: O. bicoronatus,* with two circlets of secondary caudal spines; *O. caudatus,* with three circlets; and *O. quadricoronatus,* with four circlets of secondary spines.

Ophryoscolex bicoronatus Dogiel 1927

Ophryoscolex caudatus forma *bicoronatus* Dogiel 1927, pp. 195–199, figs. 108–109; Hsiung 1931, p. 38.

Ophryoscolex caudatus forma *bicoronatus* aberration *atavus* Dogiel 1927, pp. 195, 198, fig. 109.

Diagnosis.—Body relatively stout (1.76 dorso-ventral diameters in length); long, slender main caudal spine; two circlets of caudal spines, anterior circlet composed of 6 spines, posterior circlet composed of 2 or more spines; 9 contractile vacuoles. Length 120–170μ.

Occurrence.—Dogiel (1927) reports *Ophryoscolex bicoronatus* from sheep from Sebastopol, U.S.S.R. and Hsiung (1931) reports it from sheep from China.

Relationships.—*O. bicoronatus,* like *O. bicinctus,* has only two circlets of secondary spines, but has one more spine in the anterior circlet.

Ophryoscolex caudatus Eberlein 1895

Ophryoscolex caudatus Eberlein 1895, pp. 247–250, pl. 16, fig. 4; Günther 1900, pp. 641–648, figs. 1–6; Buisson 1923*a*, pp. 129–130, fig. 48; Fantham 1926, p. 568; Becker and Talbott 1927, p. 258, fig. 26.

Ophryoscolex caudatus forma *tricoronatus* Dogiel 1927, pp. 199–202, figs. 110–111; Hsiung 1931, p. 38.

Diagnosis.—Body relatively stout (1.65–1.70 dorso-ventral diameters in length); long, slender main caudal spine; three circlets of secondary spines, anterior circlet composed of six spines; nine contractile vacuoles. Length 137–162μ.

Occurrence.—*Ophryoscolex caudatus* has been reported from sheep and cattle from Germany by Eberlein (1895) and by Günther (1900); from South Africa (Fantham (1926); from Iowa by Becker and Talbott (1927); from China by Hsiung (1931); from various parts of the U.S.S.R. and from Persia by Dogiel (1927).

Relationships.—O. caudatus, the commonest species of the genus, has three circlets of secondary spines, thus standing between *O. bicoronatus* and *O. quadricoronatus.*

Ophryoscolex quadricoronatus Dogiel 1927

Ophryoscolex caudatus forma *quadricoronatus* Dogiel 1927, pp. 202–206, figs. 112–114.

Diagnosis.—Body relatively stout (1.7 dorso-ventral diameters in length); long, slender main caudal spine; four circlets of secondary spines, anterior circlet composed of six spines; nine contractile vacuoles. Length 163μ (128–180μ).

Occurrence.—Dogiel (1927) reports *O. quadricoronatus* from domestic sheep from Bokhara, Turkestan, and from *Ovis orientalis cycloceros* from northern Persia.

Relationships.—*O. quadricoronatus* possesses the most highly developed set of posterior spines in the whole family. Its nearest relative is *O. caudatus.*

OCCURRENCE OF GENERA AND SPECIES IN *BOS INDICUS*

The same method of counting followed in the first paper of this series was used to obtain the following data. The species of ***Epidinium*** and ***Ophryoscolex*** are listed separately, the rest of the ***Ophryoscolecidae*** (exclusive of ***Entodinium***) are lumped together.

	Coonoor, India					Colombo, Ceylon				Number of hosts infected
	1	2	3	16	17	6	7	14	15	
Epidinium caudatum	....	....	....	....	....	....	....	4	8	2
bicaudatum	....	....	....	....	....	....	....	....	2	1
tricaudatum	....	....	....	..	....	....	....	....	3	1
quadricaudatum	....	....	....	....	....	....	....	....	P	1
cattanei	....	....	...	P	....	P	2	....	....	3
eberleini	...	....	2	....	1	13	....	7	....	4
Ophryoscolex spinosus	....	....	P	....	P	....	....	....	....	2
Other Ophryoscolecidae	100	100	98	100	99	87	98	89	87	

The numbers at the head of the columns refer to the original numbers of the collections.

The numbers in the columns indicate the percentage occurrence of individuals of each species. P indicates that the species is present, but in such small numbers that it was not found during the counting of one hundred individuals from each host.

This table shows clearly that *Epidinium* is rare in *Bos indicus,* particularly in comparison with the large numbers found in the European domestic cattle (*Bos taurus*) in which *Epidinium* is a very common form. Only two species of *Epidinium* were found in the hosts from Coonoor, India, while six species were found in the hosts from Colombo, Ceylon. They are also far more abundant in the Ceylonese hosts. On the other hand, *Ophryoscolex* is found only in two of the Indian hosts. These differences between the fauna of the Indian forms and the Ceylonese forms are the most striking which have been found in the whole ciliate fauna of *Bos indicus.*

HOLOTRICHOUS CILIATES

The holotrichous ciliates found in ruminant stomachs were always badly distorted by the fixing fluids used. *Isotricha,* which is an elongate, flattened organism in life, appears as a short, rotund pear-shaped form after fixation. For this reason, it does not seem worth while to give a description of the holotrichous ciliates from *Bos indicus,* since only fixed material is available.

Isotricha prostoma Stein 1859, and *Dasytricha ruminantium* Schuberg 1888 are the common ciliates of this group in the material from *Bos indicus. Isotricha intestinalis* was not found in this material. *Bütschlia parva* Schuberg 1888 is less common, and was the only species of this genus identified in this collection. *Charon ventriculi* Jameson 1925 is very rare in *Bos indicus.*

DISCUSSION

Extensive studies on the ciliates from ruminants, both wild and domesticated, by Dogiel (1921–32) and cross-faunation experiments by Becker, Schulz, and Emmerson (1930) have shown that there is very little specificity in the relationship between the ciliates and their hosts. A few genera, *Caloscolex* (from the camel), *Cunhai* (from *Cavia*), *Opisthotrichum* (from African antelope), and *Epiplastron* (from African antelope) are found only in a single species of host or in a group of closely related species of hosts. A few genera, while present in many different hosts, appear to be better adapted to one. *Ophryoscolex* and *Polyplastron* are found in both cattle and sheep, but are far more common in sheep and are only occasionally found in cattle. These few examples of restriction in host species, however, seem to be far overbalanced by the large number of cosmopolitan species and genera. Corresponding with this lack of host specificity, there has been reported little specificity in geographical distribution of the Ophryoscolecidae. Recently, however, some evidence has come up to show a certain amount of geopraphical segregation. Dogiel (1932) finds that the ciliate fauna of antelope of three regions of Africa—Congo, Uganda, and East Africa—show significant differences.

The only report on ruminant material from either India or Ceylon previous to the present series is on material from the mouse deer (*Tragulus meminna*) from Ceylon. Dobell (1910) stated that the stomach contents of this small ruminant contained many ciliates of the Ophryoscolecidae. Jameson (1925), to whom Dobell's material was given, described one new species (*Entodinium ovalis*), and noted the presence of several other species which he considered to be identical with the species from European cattle, although the forms from the mouse deer showed striking differences from the European forms in such characters as spination, shape of the body, and number of vacuoles.

The majority of ciliates from *Bos indicus* are found in other parts of the world, but a few are apparently found only in Asia. The *D. crista-galli* group (*D. laeve, D. crista-galli,* and *D. flabellum*) have been found only in Northern Persia and in India and Ceylon. *Elytroplastron bubali* is found in three different hosts (*Buffelus bubalus, Bos indicus,* and domestic sheep) all from Asia. *Entodinium bimastus* has been found in three hosts (domestic cattle, *Buffelus bubalus,* and *Bos indicus*) from Asia and European U.S.S.R. All these species of ciliates are easily recognized and have been reported at various times, so it is probable that this restriction in distribution is real and not due to insufficient reports.

These indications of natural restriction of certain genera and species, either to particular hosts, or to a particular region, in spite of a lack of any fundamental specificity of host-parasite relationships, emphasize the need of careful studies of the ciliate fauna of ruminants, both wild and domesticated, in different parts of the world. Although the ciliate fauna of widely differing types of ruminant hosts may be artificially transferred, and although such transfer is possible and probable under natural conditions, as shown by the wide distribution both in location and in number of different hosts of such forms as *Eudiplodinium, Epidinium,* etc., the more or less limited distribution of some genera and species indicates that possibilities of transfaunation are hindered by geographical barriers or by slight physiological variations of hosts which render them unsuitable for certain species of ciliates. These questions can only be settled by careful studies of many species of hosts in the same locality and the same species of hosts in widely differing localities.

SUMMARY

1. The genus *Epidinium* is restricted to those species with three skeletal plates, and a new genus *Epiplastron* erected for those with five plates. Six old species of *Epidinium* are redescribed from the material from *Bos indicus.*

2. The genus *Ophryoscolex* is represented by one species, *O. spinosus,* a new species, in *Bos indicus.*

3. Four holotrichous ciliates were identified in the material from *Bos indicus: Isotricha prostoma* Stein, *Dasytricha ruminantium* Schuberg, *Bütschlia parva* Schuberg, and *Charon ventriculi* Jameson.

4. Six species of *Epidinium* were found in the material from Colombo, Ceylon, and only two species in the material from Coonoor, India. *Ophryoscolex,* on the other hand, is found only in two of the Indian hosts. These are the only striking differences found between the ciliate fauna of the Indian hosts and of the Ceylonese hosts.

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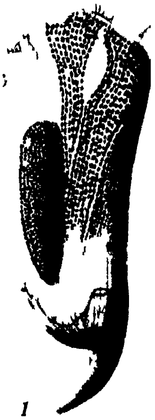
EXPLANATION OF PLATE

All figures are from whole mounts stained with chlor-zinc-iodide and drawn with camera lucida. × 500.

Fig. 1. *Epidinium caudatum* (Fiorentini 1889).
Fig. 2. *Epidinium tricaudatum* (Sharp 1914).
Fig. 3. *Epidinium quadricaudatum* (Sharp 1914).
Fig. 4. *Epidinium bicaudatum* (Sharp 1914).
Fig. 5. *Epidinium eberleini* (Cunha 1914).
Fig. 6. *Ophryoscolex spinosus* sp. nov.
Fig. 7. *Epidinium cattanei* (Fiorentini 1889).



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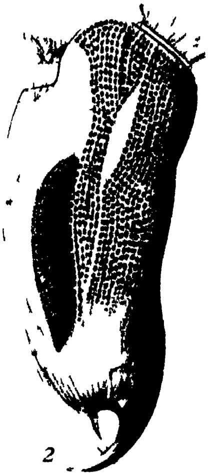

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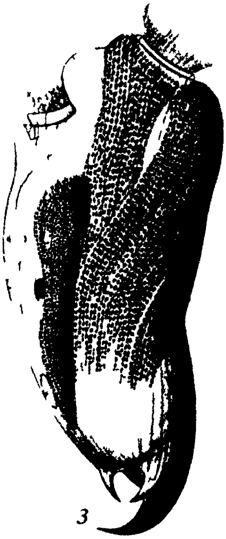

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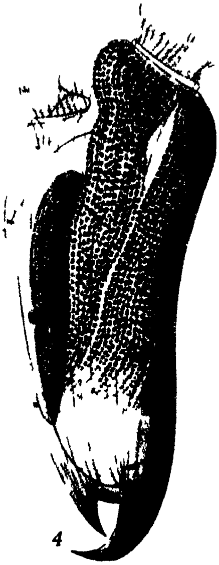

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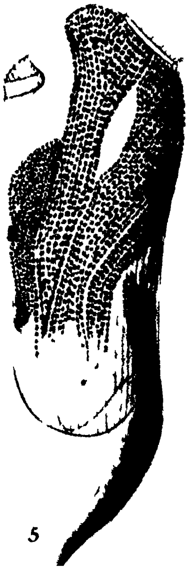

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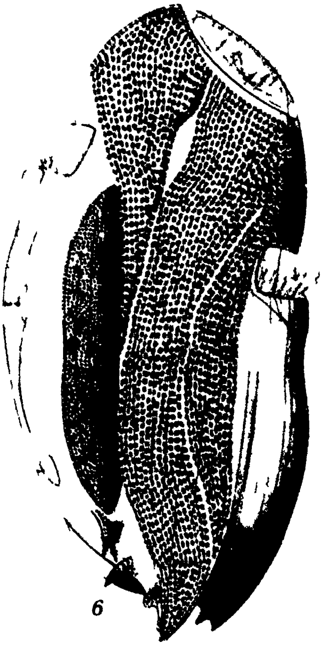

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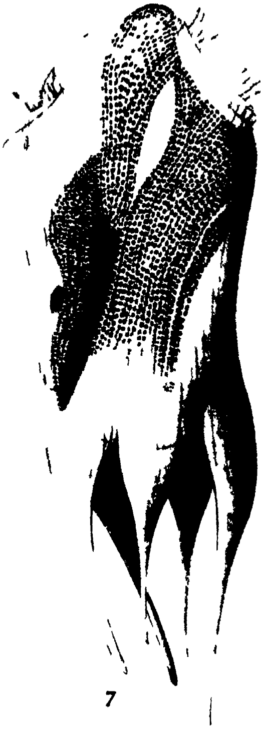

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A CORRELATION OF THE SILVERLINE AND NEUROMOTOR SYSTEMS OF PARAMECIUM

BY

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INTRODUCTION

Paramecium has been so extensively studied that one is impressed in looking over the literature with the number of techniques that have been employed to this end. Many of these methods have demonstrated structures previously undescribed, or described in a different way; and frequently no agreement has been reached on the causes of the discrepancies. There exist at this time such differences of interpretation of the fibrillar system of *Paramecium,* and it is the hope of the writer that this presentation may contribute toward harmonizing these conflicting views.

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REVIEW OF LITERATURE

The work on fibrillar structures in Protozoa has been so completely and ably reviewed by tenKate (1927) that it seems unnecessary to mention here more than those researches that have an immediate bearing on the problem of fibrils in *Paramecium.* The historical sketch of Parker (1929) gives an excellent account of the early work on neurofibrils, particularly those found in metazoan cells.

The work of Englemann (1880) laid the foundation for much of the early investigation of the mechanism of the movement of cilia. Most of Englemann's observations were, to be sure, on metazoan cells; but *Carchesium* was given some attention. Except for the excellent work of Maupas (1883), almost two decades passed without further contributions to the problem we are now considering. Three points are of particular interest in Maupas' discussion. In the pellicular pattern of *Paramecium* he described only rectangular fields; in the gullet he described an undulating membrane with a basal plate, composed of numerous rows of basal granules resting on a homogeneous basal lamella; and, lastly, he found the outer end of the trichocysts to be of a material less dense and more hyaline than their general composition. Of the first two points in particular we shall have occasion to speak later.

Heidenhain (1899) was apparently the first to demonstrate fibrils, basal granules, and cilia in approximately their correct relationship by the use of mercuric chloride as a fixing agent, and iron-haematoxylin for staining. His work, which was largely on ciliated epithelium, confirmed the results obtained by Englemann. His description of the nature of the connections between the basal granules was clearer than previous accounts, and it is noteworthy that he encountered, and mentioned for the first time, to the writer's knowledge, the great difficulty of distinguishing an actual connection from an apparent one that was effected by the close proximity of the granules.

Peter, also in 1899, by a series of experiments in cell dissection, tried to locate definitely the seat of ciliary movement, and finally concluded that the basal granule was the actual center responsible individually for the activity of its cilium.

The work of Kölsch (1902) on *Paramecium aurelia* and other Infusoria is worthy of consideration here in that for the first time the hexagonal pattern of the pellicular sculpturing was recorded. His figure of this somewhat exaggerates certain proportions, and the position of the trichocysts is entirely out of harmony with that presented by the majority of workers, since he incorrectly located them in the centers of the hexagons. Like Maupas, however, he correctly found the cilia to protrude from the centers of the pellicular polygons.

The following year Maier (1903) described the pellicle of *Paramecium* as being made up of hexagons, more regular and more realistic than those of Kölsch; and he too found the cilia arising from the centers of the dimples ("Grubchen"), a term he accredits to Bütschli and Jankowsky. Maier, in opposition to Maupas, believed the outer extremity of the trichocyst filament to stain more deeply than the rest. The work of Neresheimer (1903) on *Paramecium* yielded only negative results, contributing little.

The excellent work of Schuberg (1905) displayed a pellicular pattern of hexagons on the dorsal side of *Paramecium,* and rhomboids on the ventral side, at least in places. Furthermore, he found the longitudinal fibrils connecting the basal granules, and located the trichocysts in their correct position, in the centers of the anterior and posterior sides of the polygons. He figured a few at the corners of these fields, and it is not certain that he was incorrect in this, although it is a very infrequent occurrence, if happening at all. Schuberg offered a good description of the trichocysts themselves, having found not only their delicate connection to the outside, but the knob-like enlargement at the surface, which he believed to stain less intensely than the rest.

The observations of Brodsky (1908) on *Frontonia* are of interest because of the conclusions concerning the nature of the trichocysts, which are probably similar to those of *Paramecium.* Brodsky presented three significant points, namely: that the trichocysts were arranged in a regular pattern, figured as they are commonly believed today to be; that the substance of a trichocyst was a fluid or semi-fluid enclosed in a membrane; and that the cause of the discharge was purely chemical, but the result was accomplished in part mechanically by interaction of the trichocysts and the ectoplasm.

Khainsky (1910) working on *P. caudatum,* found the pellicular structure as Schuberg has described it, but he set forth the theory that the quadrilaterals were formed from the hexagons by stress on sides with unequal strengths. The cilia Khainsky placed correctly, but the trichocysts he found at the corners of his polygons. He figured ciliary rootlets, and stated: "Die Basalkörperchen sind nicht die Endpunkte des inneren Teiles der Cilien; von Basalkörperchern geht in Corticalplasma eine feine Fibrille ab, die nach der Brombehandlung deutlich gefärbt wird." He described two layers in the ectoplasm, and identified clearly internally discharged trichocysts. His interpretation of the mechanism differs only slightly from that of Brodsky. It is interesting to note that Khainsky followed fixation in bromine water with a 0.1 to 0.5 per cent solution of silver nitrate. This, he said, gave silver bromide that upon reduction deposited colloidal silver upon various structures. This technique so closely resembles those used at present in studies of the "silverline system," it is strange that he did not obtain similar results.

Tönniges (1914) confirmed most of the results of Schuberg and Brodsky on trichocysts, and added much concerning their origin that is not related to the problem considered in this paper.

Also in 1914 came the declaration by Sharp of a "neuromotor apparatus" in *Diplodinium ecaudatum,* a turning point in the interpretation if not in the demonstration of fibrillar systems in ciliates. He introduced into common usage for such structures two methods of fixation and staining that have since been extensively employed, namely, the Zenker-Mallory method and the Schaudinn iron-alum haematoxylin method. The following year Kofoid (Kofoid and Swezy, 1915) pointed out that the "motor apparatus" of certain flagellates indicated a broader application of the neuromotor concept than had formerly been evidenced. He states (p. 307), "Analogy to the neuromotor apparatus of *Diplodinium* as described by Sharp (1914) is suggested by the structural relations." By Sharp's methods, and others, Yocom (1918) demonstrated a neuromotor system in *Euplotes;* McDonald (1922), in *Balantidium;* Rees (1922), in *Paramecium;* Campbell (1926 and 1927), in *Tintinopsis* and *Favella;* Pickard (1927), in *Boveria;* Visscher (1927), in *Dileptus;* MacDougall (1928), in *Chlamydodon;* Lynch (1929, 1930), in *Entorhipidium* and *Lechriopyla;* and MacLennan and Connell (1931), in

Eupoterion. Taylor (1920) demonstrated conclusively, by the method of microdissection, that the system previously described by Yocom actually serves a correlating function. Gelei (1925), by various methods, such as Altmann's followed by acid fuchsin and light green, and Apathy's fixing fluid, followed by iron-alum haematoxylin, was able to bring out in *P. nephridiatum* a fibrillar system comparable in many ways with those found in other Protozoa by the workers mentioned above. It did not agree, however, with the system found by Rees, but Gelei in a later work (1929*b*) reconciled this feature by assuming the presence of both systems.

In 1922 Alverdes published the results of some investigations on ciliary movement. He forced out of *Paramecium* by pressure some material which seemed to promote the movement of cilia. This he referred to as "hyaline drops," since it appeared as such on the surface of the flattened animal. Cilia whose basal granules rested in this hyaline material beat longer and more vigorously than others merely in the culture fluid, and they were correlated in their movement. In one occurrence of animals sharing the same hyaline drop, it was observed that their cilia beat in unison. This might have been a coincidence.

Bozler in 1924 published a treatise on the food-taking organelles and the mechanism of feeding in *P. caudatum.* Much of his work was done with living animals, but the stained preparations were, for the most part, fixed in Flemming's, sectioned to 4 microns, or left as whole mounts, and stained with iron-haematoxylin. He gave an excellent general description of the gross structure of the cytostome, cytopharynx, and gullet, and worked out the ciliation of these regions, studying the movement of the different groups and what each accomplished. Bozler described for the first time a set of heavy fibrils proceeding posteriorly from the distal end of the gullet, the stomodeal fibrils, and was probably the first to get a clear conception of the blind pocket at the posterior and ventral extremity of this region. He was preceded somewhat in his description of the cytopharynx and gullet by Rees, especially, who had observed both the anterior and posterior zones, and had questioned their membranelle nature. Bozler's work is, however, somewhat more convincing than Rees's, since it is more adequately described and includes so much experimental evidence.

In his study of *Stentor,* Dierks (1926) reviewed most of the fibrillar literature, studied the ciliation of his animals carefully, and decided, as Maupas had done for *Paramecium,* that the basal granules of the membranelle were arranged as closely set points, resting or anchored in a "thick, structureless and rectangular" basal lamella. Like Heidenhain, he realized the difficulty of distinguishing between actual and apparent connections between the granules, and his decision was rendered as follows: "Die von früherer Autoren beschriebenen 'Endfadchen' und 'Basalfibrillen' sind optische Tragbilder bedingt durch die Torsion und dachziegelartige Aneinanderlagerung der Basallamellen."

Rényi (1926) attacked the problem of finding in cells the seat of ciliary movement. He disagreed with the conclusions of Peter, and insisted that the basal granules alone were not sufficient to promote movement of the attached cilia. From observations of ciliated cells of embryos he considered that possibly the mitochondria and other cell inclusions might be associated with the basal granules in that capacity. After working on *Paramecium* and *Opalina,* he accepted the "hyaline drop" idea of Alverdes seriously.

Leibermann in 1929 applied the nigrosin method of Cole to eight species of *Paramecium,* in an effort to determine whether or not pronounced differences in pellicular pattern occurred. He obtained staining results entirely similar to those of Bresslau (1921), Gelei (1925), and Klein (1926*b,* 1928) who had used other relief or surface staining methods. He concluded from his study that the differences in this respect between the species were not greater than the differences within a species, and consequently were of no diagnostic value.

Brown (1930) described the neuromotor apparatus of *Paramecium* from material fixed in Mann's osmic acid-corrosive sublimate solution and stained in thionin. A modification of Liebermann's method was used for pellicular study. His observations of the surface pattern and subpellicular structures do not differ greatly from those of earlier workers. Like Khainsky, he placed the trichocysts at the corners of the polygons. Since he used only whole mounts, and had no penetrant stain that permitted easy observation at any optical level, his system is entirely the superficial portion of what actually exists.

Recently there has become popular the silver impregnation method of demonstrating fibrils apparently of conductile nature. The method is not new, having been used by Golgi in 1875, and in quite a different technique by Ramón y Cajal in 1903. The introduction by Klein (1926*a, b*) of a much quicker, simpler, and more spectacular modification of the silver impregnation methods formerly employed undoubtedly accounts in part for the widespread application of this technique. According to this method, the organisms are fixed by drying, and the reduction of silver nitrate by reflected sunlight deposits colloidal silver on certain structures, which are referred to collectively by Klein as the "silverline system." Klein has studied this system in no less than sixty-one species of Infusoria, belonging to fifty-one genera (1926*a, b,* 1927*a, b,* 1929, 1930*a*), and on eight genera of flagellates (1930*b*); and he has written voluminously in defense of his method. Numerous other researchers, among them Gelei (1929*b*), Jírovec (1929), Lynch (1930), and Jacobson (1931), have tried Klein's technique on various Protozoa, and no results have been reported conflicting with the observations of the originator of the method. His interpretation, however, has occasioned considerable dispute.

Still more recently Gelei and Horváth (1931) have obtained good results with yet another silver impregnation method, called by them the "wet silver method," as opposed to the drying fixation of Klein. It is asserted to be equally as rapid as the dry method, and to have several advantages which, briefly enumerated, are: (1) the animals are less distorted, (2) they are more transparent, (3) the pellicle is not deeply colored, (4) the cytopharynx is impregnated throughout its entire length, and (5) not so many constant and uncontrollable factors are essential as are necessary with Klein's method. In group five fall such considerations as the position of the animals at the time of drying, the pH and salt content of the reagents, and the humidity of the atmosphere. Gelei (1932), using this method, has apparently found some of the finest structures so far known in *Paramecium* to be clearly demonstrated.

Jacobson recently (1931) has studied the fibrillar systems of a number of different ciliates, fixed and stained according to several methods. For whole mounts the organisms were frequently fixed in Zenker's (strong) or in formol osmium, and stained according to the toluidin blue method used by Gelei (1925). An alternative was the use of osmic acid vapor and opal blue. Klein's method was also employed. For sectioning, the material was fixed in Schaudinn's or mercuric chloride solution (both hot and cold), or mixtures of Zenker's and Flemming's fluids. The Protozoa were cut at thicknesses from 1 to 20 microns, and stained with Heidenhain's haematoxylin, Mallory's, or by the gold chloride method of Apathy. On the results of these techniques Jacobson has considered, and seriously criticized in places, the interpretations of the fibrillar structure of *Paramecium* given by Rees, Klein, and Gelei. We shall find it necessary to refer to this article on many occasions as we continue.

THE PRESENT STATE OF KNOWLEDGE OF THE FIBRILLAR SYSTEM OF PARAMECIUM

In order to bring before the reader as clear a picture as is possible of the fibrillar structure of *Paramecium* as it is known from the literature to date, there is presented below a composite summary of the results obtained by previous investigators.

The surface of *Paramecium* has on it a pattern made up of adjacent polygons, mostly hexagons, although on the ventral side, especially in the region of the mouth, anal opening, and sutures, they may be irregular. Quadrilaterals are the most common departure from the hexagonal design. It is possible that the area of irregularity of pattern is greatly increased by drying, shrinkage, and other unnatural physical conditions. In *P. nephridiatum* as described by Gelei (1925) rhomboids apparently predominate. Klein (1926*b*, 1931) is of the opinion that this pattern represents an "indirectly connected" conductile system. Gelei apparently believed at one time that the lines represented actual fibrils, but of a supporting nature, for he said (1929*a*, fig. 1, p. 276): Unter der Pellicula folgt die Skelettfibrille (Silberlinie von Klein) und darunter der neuroplasmatische Strang von mir.'' But in a later work his stand on this is doubtful, for he stated (1929*b*, p. 187): ''Dieses indirekte System ist nicht weiteres als altbekannte pelliculäre Gittersystem der Ciliaten, das als Skeletgitter zur Bewahrung der statischen Zustände des Korpers dient und mit der Reizleitung gar nichts zu tun hat.'' Jacobson (1931, p. 62) expressed a similar opinion regarding the surface pattern, stating: ''Nach den Ergebnissen von L. Peschowskaja (1928) würde auch *Paramecium* in diese Grupp gehören, da nach ihren Beobachtungen die Fasern des direkt verbindenden System die Basalkörner nicht verbinden, sondern in einem anderen Niveau seitlich neben ihnen verlaufen.''

From the depressed centers of the pellicular polygons protrude the cilia, each of which has at the proximal end a basal granule imbedded in the ectoplasm. These basal granules are connected by longitudinal fibrils, so that those in each row of polygons give the effect of a string of beads. Klein (1926*b*, 1931) and Gelei (1932) have found that in some specimens cilia are equipped with groups of two or three granules at their bases instead of the single basal granules usually described. Moreover, Gelei claims that frequently the basal equipment of a cilium is a ring and not a solid granule, as is commonly accepted. Often, he says, in *P. caudatum* two cilia arise together in the center of the same polygon, especially in the region of the oral groove. Brown has described transverse ''commissural neurofibrils'' as being typically present in *P. caudatum*, and Gelei has found similar structures in *P. nephridiatum*. Other investigators believe the presence of these fibrils to be exceptional.

Each trichocyst is fastened by a fine filament to the pellicle, where the outer end is marked by a slight enlargement. There is disagreement over the question whether they are otherwise free or are connected to the longitudinal body fibrils. There is even more disharmony regarding their position of attachment to the pellicle, and the pattern they present. Most workers find them attached either at the corners or in the centers of the anterior and posterior sides of the hexagons. Recently, the latter view seems to be most generally supported. In this view their general pattern is one of longitudinal rows converging near the sutures. Rees found two ''whorls'' of trichocysts, which he thought crossed the longitudinal ridges between the rows of cilia in such a way as to produce the effect of the geometric pattern described by others.

Most of the other fibrils have been described in the region of the cytopharynx and gullet. The ''oral membranelle'' that predominated in the literature for such a long time has recently been found to be a great number of free cilia forming a thick mass which starts on the vestibule, or outer part of the cytopharynx, and is continuous back to the fore part of the gullet. According to

Bozler it is continuous, although somewhat modified, as far as the distal opening of the gullet, just before the blind pocket is reached. It has been described as present in various places, such as along the dorsal wall, slightly to the left of the dorsal wall, and on the extreme left wall of the cytopharynx (Gelei and Horváth). All writers are so positive in their descriptions that one wonders if they observed and have reference to the same structure. The best investigations now seem to indicate that there is a sparse ciliation in the outer part of the cytopharynx and in the cytostome, and also on the ventral wall of the cytopharynx. A brush-like thickening occurs at the extreme posterior end of the gullet, but concerning its exact position there is little agreement. Usually a broad longitudinal band that exists on the left wall is considered to be free from cilia.

Bozler (1924) and Gelei (1925) contend that there proceed posteriorly from the inner extremity of the gullet a number of long fibrils, estimated at from six to ten, as well as additional shorter ones that dwindle in size until the limits of optical resolution are reached. These fibrils are usually more or less lax and intertwined in stained material, but in exceptionally well preserved specimens they are said by Bozler to be straight and parallel, forming a toboggan-slide-like structure down which the food vacuoles slide. Their point of origin has been variously ascribed. It is generally agreed that some are attached to the dorsal wall of the gullet, and others, according to Bozler, connect on the right side. They end without attachment posteriorly, and Bozler believes that they all lie in one plane, forming a flat chute.

Some declare that the wall of the cytostome is smooth, but others think that it too shows a pellicular pattern. Gelei (1925) found in the vestibule and esophagus a network of subpellicular fibrils he thought to be supporting, and elastic in nature. These spread outward in the cytostome and become connected with the general body surface, especially on the left and posterior boundaries of the oral opening. He figured this network as composed of parallelograms, each surrounding a pair of cilia (in *P. nephridiatum*).

Khainsky (1910) and Brown (1930) have described and figured ciliary rootlets from the basal granules of *Paramecium*. Usually none have been found. Similar structures were described by Rees (1922) as curving gracefully inward in two great whorls to converge toward a point in the ectoplasm anterior to the cytostome. It was here that he found the "neuromotor center," but as to the actual connection between it and the peripheral fibrils he was not clear. Jacobson (1931) believes the peripheral fibrils of Rees to have been internally discharged trichocysts, and offers considerable evidence for her view. She points out that Rees remarked that the whorls of trichocysts had courses coinciding with the neuromotor fibrils.

Besides the double body described by Rees as a neuromotor center, Gelei (1925) has described in *P. nephridiatum* two nerve centers. One of these, a bilobed mass of small size, lies on the dorsal side of the cytostome, and corresponds to that found by Rees. The second, or "posterior nerve center" of Gelei, lies very near the caudal end of the gullet, also on the dorsal side. It is inadequately described. Both investigators found several fibrils ramifying from their respective neuromotor centers into the endoplasm. Neither seemed positive of the fate of the distal ends of these structures, but Gelei left them free in the cytoplasm.

The exhaustive experimental work of Bozler (1924) is not within the scope of our problem, so the writer merely suggests that those interested may find in it some substantiation of the morphological evidence reviewed above.

The reader wishing a concise review of the most recent speculations on the origin and method of performance of the conductile elements and the neuromotor system of *Paramecium,* is referred to the summaries of Klein (1927) and Gelei (1929*b*). The writer doubts that they are based entirely on sound morphological or experimental evidence.

MATERIALS AND METHODS

Throughout these investigations *Paramecium multimicronucleata* has been used exclusively. Identification was made by consulting the descriptions given by Powers and Mitchell (1910) and Wenrich (1928). The original material was wild stock obtained from the vicinity of Berkeley, California. This was grown on ordinary hay infusion until a heavy culture resulted. A few individuals of large size were selected and transplanted to fresh hay infusion. As soon as a culture was secured in which *P. multimicronucleata* was the predominant form, it was easy by a series of subcultures to obtain one free from other species of *Paramecium* or other large Protozoa. For some of the work pure line cultures were used, grown in the medium described by Rosenberg (1932). The results of these two methods were practically identical.

The living, unstained animal is not especially helpful in the study of the fibrillar system, except in observing the movement of the cilia, particularly those of the cytopharynx and gullet, and the formation and passage of food vacuoles. In exceptional cases the pellicle can be studied in the living animal, but it is necessary to fix and stain the animal to demonstrate any of the finer fibrillar structure. Because differences do exist when different techniques are used, and since this is essentially a correlation problem, several distinct methods were employed.

Both whole mounts and sections were used. Some whole mounts were fixed in Zenker's and stained in Delafield's, Heidenhain's or Ehrlich's haematoxylin stains, or fixed in Schaudinn's and stained with Heidenhain's iron-haematoxylin. Others were treated according to the silver methods of Klein (1926*a*, *b*), Gelei and Horváth 1931), or the Yabroff modification (1928) of Da Fano's technique. Sections were of material fixed in Zenker's and stained in Mallory's triple connective tissue stain, or of material fixed in hot Schaudinn's and stained in Heidenhain's haematoxylin. A wet silver method like that of Gelei and Horváth was tried, but proved unsatisfactory. Since only four of the above methods, Zenker's with Mallory's, Schaudinn's with Heidenhain's haematoxylin, Klein's, and the method of Gelei and Horváth, gave good results, they will be discussed in more detail, and the others abandoned. The animals were concentrated by centrifuging, and, unless otherwise stated, they were fixed, washed, and dehydrated also by this method in centrifuge tubes.

Zenker's was used with 2.5 and 5 per cent acetic acid, the former proving the more satisfactory. In some treatments the living Protozoa were injected into pieces of frog's intestine, which were then tied like miniature sausages, placed in Zenker's, and then washed, dehydrated, and imbedded. It was a little more satisfactory to fix first in centrifuge tubes, and use the gelatin capsule method of imbedding. Sections were cut at 2.5, 5, 7.5, 10 and 15 microns, but 7.5 microns proved to be the most useful. The best results were obtained by staining 2 minutes in Mallory No. I and one minute in No. II, then dipping rapidly into 95 per cent and absolute alcohol, blotting quickly between each change, and then clearing in xylol for about 3 minutes.

Schaudinn's fixing fluid was also tried with 2.5, 4 and 5 per cent acetic acid. The first seemed to fix the cytoplasm the best, but the most satisfactory preparations of the fibrillar system happened to be among those fixed with the last concentration. The difference was not great. In two attempts the organisms were treated with a 0.5 per cent solution of tannic acid before fixation in Schaudinn's. It was hoped that the absence of trichocysts in the ectoplasm might aid in demonstrating some of the subpellicular fibrils. Internally discharged trichocysts and abnormally large contractile vacuoles complicated matters considerably, and the method was discarded. The capsule method of imbedding was used, and sections were cut as mentioned above. As before, the 7.5 micron sections were the most useful. Staining was by the short iron-alum haematoxylin method. The material was differentiated in 0.5 per cent cold iron alum, under constant observation by means of a water immersion objective. With experience, in properly selected sections, the fibrils themselves may be watched; otherwise the usual method of waiting for the cytoplasm to become light gray, while the macronucleus and trichocysts remain dark, will give an approximate indication of the progress of the reaction.

Klein's method is suitable only for whole mounts. It was used as described by Klein (1926*a*; *b*, footnote, pp. 243, 244) with only such variations as were found necessary for *Paramecium*. As Klein suggested, much concerning the length of time required for impregnation and reduction had to be learned by experience. The writer found that different effects could be obtained, and different structures emphasized, by varying also the degree of drying. Every modification suggested by Klein, except the use of osmic acid, was tried.

The wet silver method of Gelei and Horváth (1931), with such modifications as were especially recommended for *Paramecium,* was used. The best results were obtained by the procedure outlined below.

1. Protozoa were concentrated by centrifuging.
2. Fixed 3 minutes in a mixture of 95 c.c. concentrated aqueous solution of $HgCl_2$ and 5 c.c. formalin.
3. Washed twice in filtered river water.
4. Impregnated 3 minutes in 2 per cent solution of $AgNO_3$.
5. Without washing, reduced in distilled water by direct sunlight for 8 minutes. Here the centrifuge tube was fixed in a position so that the sun, at noon, shone vertically into the tube. A fine capillary tube was passed to the bottom of the container, and the air was bubbled through the water to keep the animals dispersed and rotating, thus securing more uniform reduction.
6. Washed five times in distilled water.
7. Dehydrated slowly. Into 1 c.c. of distilled water containing the animals was passed 9 c.c. of 95 per cent alcohol, slowly, by allowing it to filter through three sheets of No. 1 filter paper. Again a fine stream of air introduced through a capillary tube kept the mixture uniform.
8. Passed through absolute alcohol, cleared in xylol, and mounted. This was done by placing a drop of xylol in the center of a one-half inch ring of balsam, as prescribed by Gelei and Horváth for keeping the animals in the center of the slide.

No advantage was found in toning with gold chloride.

MORPHOLOGICAL ASPECTS

Since the two groups of methods, one dependent upon the reduction and deposition of colloidal silver, the other upon the affinity of the structures for soluble stains, gave quite different results, the writer presents below the descriptions of the two systems separately. With a clear picture of each system in mind, the correlation which follows will be much easier to understand.

THE SILVERLINE SYSTEM

The silverline system was briefly defined by Klein (1927*a*) in this way: "Im Ciliatenkörper lässt sich mit der später hier gegebenen einfachen Versilberungsmethode, das rein ektoplasmatisch, in der Höhe der Basalkörner verläuft und sich durch Silber tief braunschwarz imprägniert. In diesbezüglichen Präparaten treten die Fibrillen dieses Systems als scharfbegrenzte braunschwarze Linien auf, die durch Silber dargestellt wurden; deshalb und weil diese Systeme im ganzen und in ihrem Zusammenhange mit gleichreagierenden Organellen durch andere Methoden nicht darzustellen sind, benannte ich diese Fibrillen 'Silberlinien' und die Komplexe derselben 'Silberliniensysteme'." This system the writer has demonstrated, and compared with the results of Klein in thirteen genera of Infusoria, to be sure the technique was perfectly duplicated. Results obtained did not differ from those of Klein except in minute details.

The silverline system of *Paramecium* consists of two parts. One portion is made up of a series of closely set polygons, usually hexagons, but flattened into rhomboids or other quadrilaterals in the regions of the cytostome, cytopyge and sutures. Where hexagons occur they appear in longitudinal rows, with a side at the anterior and posterior extremities, and a lateral vertex to each side (pl. 5, figs. 12, 14; pl. 6, fig. 18; pl. 7, fig. 25). Where quadrilaterals occur they too may appear in longitudinal rows, lapped to about half their length with those of adjoining rows, like bricks in a wall. Great irregularities frequently occur, however, the lap being lost first, and then even the arrangement in rows. This is especially pronounced in the cytostomal region (pl. 5, fig. 11).

This system of lines stains intensely if the animals are quite thoroughly dried, appearing very dark brown against the golden or straw-colored background. Usually the lines appear solid (pl. 5, fig. 12), but frequently they are interrupted to seem double at the vertices of the polygons (pl. 6, fig. 18; pl. 7, fig. 25). Klein has emphasized this effect in interpreting this system as an "indirectly connected" conductile system (1928, 1931). One of his figures (1928, fig. 6, p. 196) shows clearly the extent to which this apparent double nature of the polygons can be found, if carefully sought. In the centers of the anterior and posterior sides of the hexagons, or rectangles, are located one or, rarely, a cluster of two to four darkly staining granules, which mark the outer ends of the trichocysts, i.e., their attachment to the pellicle. Irregularities, loops, and transverse bridges occur occasionally in this system (pl. 6, fig. 18; pl. 7, fig. 25). The entire system is poorly demonstrated or entirely lost if the animals are not completely dry on the surface before the silver nitrate is added. The trichocyst granules are quite persistent in taking the silver.

The second part of Klein's silver line system comprises what he termed the "directly connected" conductile system (1926*b*, 1928, 1931). It consists essentially of the longitudinal lines linking into a chain all the basal granules in one longitudinal row of hexagons—or quadrilaterals, and of the basal granules themselves, which are at or near the centers of the polygons of the first portion (pl. 5, fig. 12; pl. 6, fig. 18; pl. 7, fig. 25). Frequently there appear two, three, or, more rarely, four granules instead of one, and of these Klein (1928, fig. 6, p. 196) designates one as a basal granule, and the others he calls "Nebenkörner," which presumably function as secondary or accessory basal granules. These, and other granules with neither names nor supposed functions, are always connected by delicate strands to either the longitudinal lines or the hexagonal figures, or both (pl. 6, fig. 18; pl. 7, fig. 15). Much variation occurs among these "accessory" granules or lines, and it is the writer's conviction that certain of the apparent granules, particularly the smaller ones, are merely deposits of silver at points where two extremely fine fibrils intersect or cross.

There is also an occasional occurrence in almost every individual of a third element, namely, transverse fibrils, which pass from one basal granule to another nearby, in one of the adjacent rows (pl. 5, fig. 12). In the region of the mouth such transverse fibrils have been found with such regularity that they doubtless represent portions of a set of radial cytostomal fibrils, which will be considered later. They are fine, not consistently present, or failing often to hold the silver, and are among the first to disappear as the preparation loses its color. Unfortunately, they cannot be demonstrated in thoroughly flattened and dried specimens, which retain their color the best, but are apparently broken or distorted.

With the exception of the radial fibrils, this entire system usually stains more intensely than the system of polygons first described. In animals which have not been thoroughly dried before immersing in silver nitrate, only the longitudinal lines and the basal granules are clearly demonstrated; the pellicular polygons can be made out only with the greatest difficulty. When both are clearly visible, this fraction of lines and basal granules can be observed to lie at a slightly lower level than the first. The difference is not great, but enough so that even in a photomicrograph the discrepancy in focus of one or the other system can be detected in many animals (pl. 7, fig. 25).

The cilia of *Paramecium* can frequently be seen very clearly, but the writer has been unsuccessful in duplicating the results of Klein (1929*a*) on their finer structure.

It seems best to discuss at this time also the results obtained by the wet silver method of Gelei and Horváth (1931), even though it is necessary in doing so to remember that they do not fall strictly within our previous definition of the silverline system.

With this wet silver method the pellicular and subpellicular portions of the peripheral system can again be distinguished, and, in addition, the portion associated with the cytopharynx and gullet can be demonstrated. As before, the system of pellicular polygons is the fainter of the two peripheral portions; so much so, in fact, that often it can scarcely be found. The points of attachment of the trichocyst bodies are very distinct. Irregularities, and the apparent double make-up of the lines, are either far less pronounced or entirely absent.

The subpellicular peripheral portion coincides very closely with that found by Klein's method. The basal granules seem more frequently to have accessory granules, or to be multiple. The writer is convinced that the basal ring described by Gelei (1932) is either an effect produced by viewing in optical section the outer deposit of silver on one large granule or a cluster of gran-

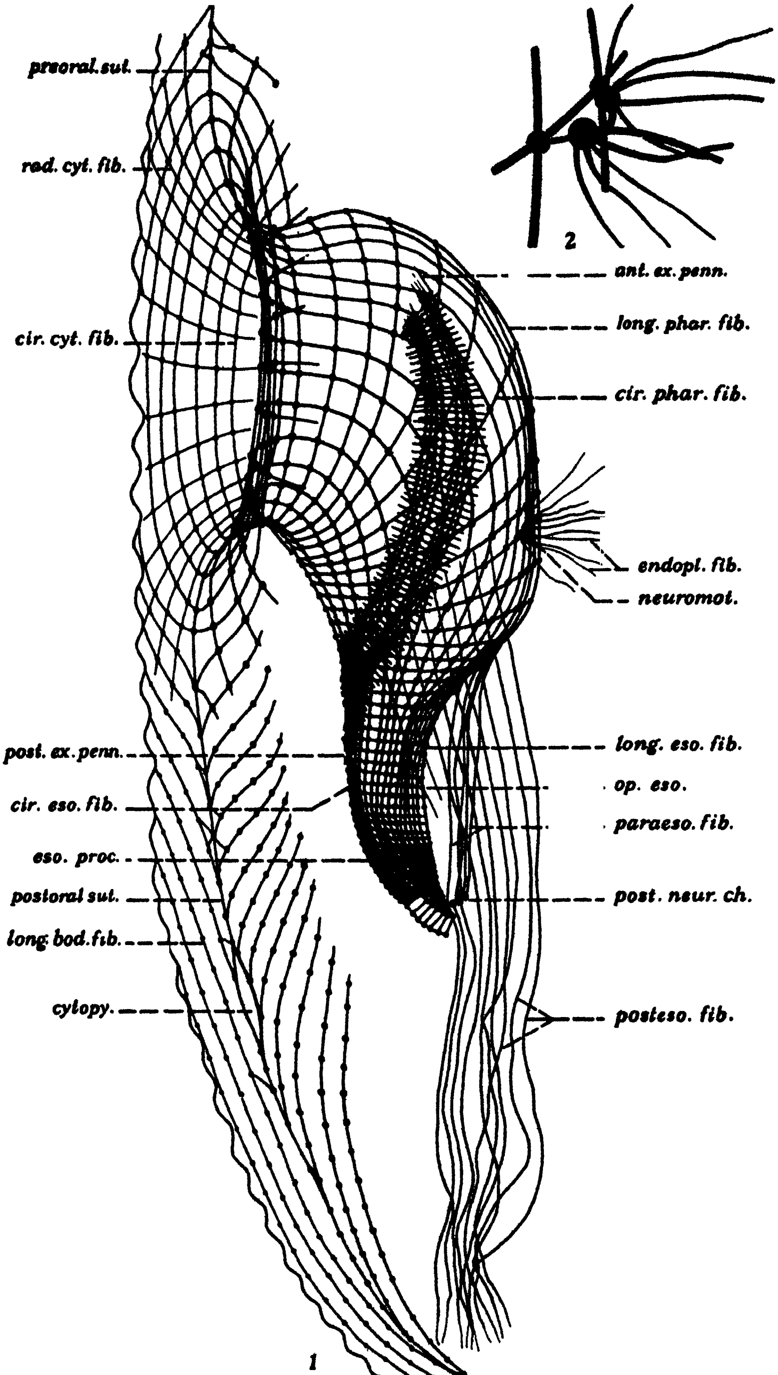

Fig. A. 1. Diagrammatic representation of the pharyngo-esophageal network, with its associated fibrils of the body surface and the endoplasm, in *P. multimicronucleata*. The cilia have been omitted for the sake of clearness. × 2250. *ant. ex. penn.*, anterior extremity of penniculus; *cir. cyt. fib.*, circular cytostomal fibril; *cir. eso. fib.*, circular esophageal fibril; *cir. phar. fib.*, circular pharyngeal fibril; *cytopy.*, cytopyge; *endopl. fib.*, endoplasmic fibril; *eso. proc.*, esophageal process; *long. bod. fib.*, longitudinal body fibril; *long. eso.*

ules, or, as is more probable, that the ring is made up of two to four granules with connecting fibrils, as demonstrated by Klein's method (pl. 6, fig. 18; pl. 7, fig. 25). The longitudinal fibrils do not seem always to follow a zig-zag path, as Gelei (1932) found them to do, but do so occasionally. It is possible that many of the larger basal granules, particularly in the cytostomal region, as found by Klein's method (pl. 5, fig. 11) have the same multiple composition as is shown so frequently by the method of Gelei and Horváth, but are covered by a relatively heavy deposit of silver.

This entire subpellicular portion stains far more heavily than the system of polygons first described, and lies definitely below the former. Since the animals are neither dried nor flattened, and only slightly distorted, the true relative positions of two portions are more nearly approached, and more readily distinguished as being in separate planes than by Klein's method.

The system of fibrils in the region of the cytostome and gullet is not demonstrated by Klein's method, but has been seen clearly in whole mounts prepared by the wet silver method. Gelei (1932) described pellicular ridges in the walls of the cytopharynx, found basal granules far into the gullet, and described and figured in a photomicrograph the "Penniculus." Of this he says (p. 162): "Wie steht es nun mit den Relatoren in der Membranelle von *Paramecium* in meinem Penniculus (1925), der in Cytopharynx links gelagert ist und der gewohnlich aus acht Cilienreihen aufgebaut ist? (Taf. 5, fig. 5)."

The writer finds that the pellicular ridges extend far in toward the gullet, and present a figure of a series of trapezoids with their shorter bases directed inwardly toward the gullet. In the center of each is a basal granule, thus making a pattern very similar to that of the general body surface. The basal granules are connected anteriorly and posteriorly by the longitudinal pharyngeal fibrils, and laterally by circular pharyngeal fibrils which circumscribe the cytopharynx, upper gullet, and esophageal process. The outer ends of the longitudinal pharyngeal fibrils spread out in the area about the cytostome, forming the radial cytostomal fibrils, whereas the circular pharyngeal fibrils have their origin outside in the converging longitudinal body fibrils (fig. A). The penniculus is just as Gelei described it, and in *P. multimicronucleata* consists always of eight parallel rows of basal granules, all approximately equally spaced except that a slightly greater distance is present between the fourth and fifth longitudinal rows. Transverse fibrils are also present (fig. A).

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fib., longitudinal esophageal fibril; *long. phar. fib.*, longitudinal pharyngeal fibril; *neuromot.*, neuromotorium; *op. eso.*, opening of esophagus; *paraeso. fib.*, paraesophageal fibril; *posteso. fib.*, postesophageal fibril; *post. ex. penn.*, posterior extremity of penniculus; *post. neur. ch.*, posterior neuromotor chain; *postoral sut.*, postoral suture; *preoral sut.*, preoral suture; *rad. cyt. fib.*, radial cytostomal fibril.

2. Diagrammatic representation of the neuromotorium, in approximately the position figured in A. 2. × 9000.

Since all the structures described above from the region of the gullet had been seen much better in specimens prepared by other methods before the wet silver method was used successfully, a complete description will be given later as the results of these methods are discussed.

THE NEUROMOTOR SYSTEM

The term "neuromotor apparatus" was used by Sharp (1914) to designate a complex of fibrils in *Diplodinium ecaudatum.* He attributed to them a conductile function for the following reasons: all parts of the structure were connected; portions of it supplied every movable part of the protozoan body; all these bodily parts moved with coordination; and the size and manner and position of attachment of the fibrils themselves did not indicate a supporting or contractile function. For similar reasons numerous other researchers previously mentioned have come to similar conclusions concerning integrated systems of fibrils in other Infusoria, and the writer likewise describes the system found in *P. multimicronucleata.* Any additional evidence for its identity as a conductile system, or comments bearing on this evidence, will be considered in the discussion which follows this description. Since the two expressions have been used interchangeably, the writer has chosen "neuromotor system" in preference to "neuromotor apparatus," because he believes it to be more indicative of the extent and intricacy of the entire complex.

In considering the neuromotor system of *Paramecium,* the writer will first discuss the nature of the pellicle and its associated parts, which are not parts of the system at all, but must be kept in mind for purposes of orientation; then the peripheral or subpellicular portion, which is truly neuromotor; and finally the various parts of the complex pharyngeal portion.

Delafield's and Heidenhain's haematoxylin and Mallory's stain all show the surface of *Paramecium* to be made up of a series of polygons, which are hexagons everywhere except in the regions of the sutures, cytostome, and cytopyge. Here the pattern is often greatly disturbed. The basal granules, which serve as valuable landmarks, are always at or near the centers of the polygons, and slight enlargements in the centers of the anterior and posterior sides of the hexagons mark the distal ends of the trichocysts. These seldom if ever occur elsewhere (pl. 5, fig. 12). Sections up to 7½ microns in thickness stained with Heidenhain's iron-haematoxylin show especially well the contour of the surface (pl. 5, fig. 13; pl. 3, figs. 2–6; pl. 4, fig. 10). The walls of the hexagons are ridges, surrounding depressions or dimples slightly less than one micron in depth, while the diameter of the hexagons from ridge to ridge varies from about 1.8 to perhaps 6 or more microns. There appears to be no significant correlation between body size and the size of the hexagons. To the centers of the anterior and posterior walls of the polygons are attached trichocysts.

These carrot-shaped bodies, about 4 or 5 microns in length, are between 1 and 2 microns below the surface, to which each is attached by a delicate thread. The writer does not believe them to be otherwise connected, and hence not a part of, nor associated directly with, the neuromotor system. This conclusion is based on the ease with which the trichocysts are moved, apparently swinging freely from their pellicular attachment. If they are connected with the longitudinal body fibrils, as suggested by some investigators, the union is extremely delicate and fragile.

Cilia of different lengths, dependent upon the individual and the part of the body considered, project outward from the basal granules, thus protruding from the centers of the pellicular polygons. In *P. multimicronucleata* there is only one cilium to each hexagon. The basal granules are connected in longitudinal chains, corresponding with the rows of pellicular polygons, by the longitudinal body fibrils (fig. A, *1, long. bod. fib.;* pl. 4, figs. 7, 8; pl. 5, figs. 12–14). Over most of the body these run quite straight, from pole to pole; but in the region of the sutures those on the left side particularly curve sharply inward. The pattern at the poles has been figured by almost every recent investigator of *Paramecium,* and will therefore be omitted. It is interesting that the cytostomal region the fibrils of the right side also curve inward, so that the right and left sides form complete circuits about the cytostome, the circular cytostomal fibrils (fig. A, *1, cir. cyt. fib.;* pl. 4, fig. 7; pl. 5, fig. 11). Except in this region, however, the longitudinal fibrils of the right side proceed almost parallel to the sutures, which are made up in part from these fibrils. The cytopyge is a permanent structure, the postoral suture being divided to accommodate it (fig. A, *1*). Near the mouth and cytopyge transverse bridges and other irregularities frequently occur along the sutures, especially along the postoral one. Usually a variable number of fibrils, often between forty and fifty, radiate outward from the cytostome, connecting transversely the basal granules of the surrounding rows. It is convenient to call these the radial cytostomal fibrils (fib. A, *1, rad. cyt. fib.;* pl. 4, figs. 7, 8). They are longest and most easily seen on the left anterior margin of the cytostome, but even there they pass outward only a short distance. Occasionally transverse bridges may appear even on the dorsal side of the animal (pl. 5, fig. 12), but in all individuals they seem exceptional, thus presenting a system quite different from that described by Pickard (1927) in *Boveria,* and that of *Eupoterion* described by MacLennan and Connell (1931). Ciliary rootlets, or anything comparable to them, have never been found. Internally discharged trichocysts frequently give an effect that might be mistaken for the presence of ciliary rootlets or other internal connections. If carefully studied these may usually be positively identified as trichocysts by the positions of their outer ends, and by the condition of the surrounding trichocyst bodies (pl. 5, fig. 15).

THE PHARYNGEAL COMPLEX

Considerably more complex than the part of the neuromotor system already described is that portion associated with cytopharynx and gullet, or esophagus. It consists of seven related parts, some of which have several components within them. These parts are: (1) the pharyngo-esophageal network, (2) the neuromotorium, and associated fibrils, (3) the penniculus, (4) the esophageal process, (5) the paraesophageal fibrils, (6) the posterior nuromotor chain, and (7) the postesophageal fibrils.

The pharyngo-esophageal network is a trumpet-shaped structure lying in the walls of the cytopharynx and gullet (fig. A, *1*). It is made up of the basal granules of the cytopharynx and esophagus, the longitudinal pharyngeal and esophageal fibrils (fig A, *1, long, phar, fib.*, *long. eso. fib.*, pl. 2, fig. 1; pl. 3, figs. 3–6; pl. 4, figs. 7–10; pl. 6, figs. 16, 17, 19–21; pl. 7, figs. 23, 24, 27–29) which are the inner portions of the radial cytostomal fibrils (fig. A, *1, rad. cyt. fib.*), and the circular pharyngeal and esophageal fibrils (fig. A, *1, cir. phar. fib.*, *cir. eso. fib.;* pl. 1, fig. 1; pl. 3, figs. 3–6; pl. 4, figs. 7–10; pl. 6, figs. 16, 17, 19–21; pl. 7, figs. 23, 24, 27–29). These circular fibrils of the cytopharynx and esophagus correspond to the circular cytostomal fibrils (fig. A, *1, cir. cyt. fib.*) which were observed to have their origin in the converging longitudinal body fibrils. Thus it is seen that the longitudinal fibrils of the general body surface become the circular fibrils of the pharyngeal region, whereas the longitudinal fibrils of the pharyngeal region become the radial fibrils of the body surface. The intersections of the fibrils of each set with those of the other are marked by basal granules, and from most of these granules, perhaps from all, cilia protrude into the cavity of the cytopharynx and esophagus (pl. 3, fig. 5; pl. 4, figs. 7–9; pl. 6, figs. 17, 20, 22; pl. 7, figs. 23, 24, 27, 29). Not all the cilia are of the same length. There are regularly some prominent cilia on the left wall of the cytopharynx, but they are difficult to separate from the ciliation of the penniculus, which will be discussed later. The right wall also has a narrow band of rather long cilia, but they are not numerous and cannot always be demonstrated (pl. 4, figs. 7–9; pl. 6, fig. 22; pl. 7, fig. 29). Except the dorsal wall most of the rest of the cytopharynx and esophagus has either exceedingly short, fine cilia, or none at all. The dorsal cilia are long and heavy, forming a continuous band throughout the posterior two-thirds of the cytopharynx, and as far down the esophagus as its dorsal wall is intact. Contrary to the usual observations, however, the esophagus does not expel the food from its posterior extremity, but from an opening on its dorsal side, leaving beyond that a blind pocket, the esophageal process.

On the left dorsal wall of the cytopharynx, at about the level of the posterior margin of the cytostome, is a very small, bilobed mass, the neuromotorium (*fig. A, 1, neuromot., 2; pl. 3, fig. 4; pl. 6, fig. 16; pl. 7, fig. 23*). Each lobe is slightly larger than the basal granules of the pharyngeal region, and is connected by at least one, and possibly two short, fine fibrils to one of these near-by basal granules. In some specimens a considerable space between the two lobes permits one to see a connecting fibril, in others the two granules lie adjacent, and many specimens either reveal no such bodies or they are too well concealed by other structures to be studied, even with difficulty. From the neuromotorium a number of fibrils radiate out into the endoplasm. Of these, four or more usually pass almost to the dorsal body wall, but the rest, which seem to be variable in number, are shorter and not definite in position. All are termed endoplasmic fibrils (fig. A, *1, endopl. fib.*, *2;* pl. 2, fig. 1; pl. 3, fig. 4; pl. 4, fig. 10; pl. 6, fig. 19; pl. 7, fig. 26). Approximately half of the endoplasmic fibrils come from each granule. Their function is unknown, but they may coordinate the feeding movements of the oral cilia with the lack or abundance of food in the endoplasm, thus serving a nutrition-regulating function. Their distal ends are unattached.

It is difficult to identify the neuromotorium and the endoplasmic fibrils described here as being those found and figured by Rees (1922, pl. 35, fig. 22), for neither his description nor his illustrations are sufficiently clear. Both the bilobed body and its accompanying fibrils are without doubt identical with the structures described and figured by Gelei (1925, fig. 8), who referred to them as the "Vorderes Nervenzentrum und seine Verästelungen." Since this body resembles both in structure and in position the neuromotoriums described in other Infusoria, and is intimately associated with both the ectoplasmic and endoplasmic fibrils, the name "neuromotorium" is appropriate.

The penniculus (fig. A, *1, penn.;* pl. 2, fig. 1; pl. 3, fig. 4; pl. 6, figs. 16, 22; pl. 7, figs. 23, 29) is composed of eight rows of basal granules, four equally spaced rows being separated by a slightly greater gap from four more equally spaced rows. The basal granules, usually numbering over sixty to each longitudinal row, are connected by both longitudinal and transverse fibrils. The ends of these fibrils of both sets extend a short distance beyond the last basal granules, and end apparently without specific attachment. The entire structure lies slightly deeper than the pharyngeal network, and proceeds from the anterior, left dorsal wall of the cytopharynx, around the left wall in a slightly oblique direction to terminate on the anterior, ventral wall of the esophagus. Since each basal granule bears a cilium, a heavy band of cilia results. The connection of the penniculus to the pharyngo-esophageal net is not known, but the writer believes that the cilia of the penniculus beat independently of the rest of the pharyngeal cilia, aiding indirectly the passage of food into the esophagus, while those of the lower end help in forming food vacuoles. The idea that the cilia of the penniculus are independent is not new, for Gelei (1925, p. 160) says: "Der Peniculus ist ein spezifisches Schlundorgan; ob die Cilienreihen selbständig in dem Schlund auftreten, oder ob sie Fortsetzungen der entsprechenden vestibularen Cilienreihen sind, konnte nicht entscheiden werden." But in the copy consulted by the writer Gelei had added after this, in the margin, "Doch! sie sind selbständige Schlundorgane."

At the posterior extremity of the gullet the circular fibrils once more completely enclose the cavity, so that a tube results. Furthermore, the longitudinal fibrils converge at the posterior end and unite in a double row of granules bound by transverse fibrils, so as to close the end of the tube, making a blind pouch, the esophageal process (fig. A, *1 eso. proc.;* pl. 2, fig. 1; pl. 4, fig. 10; pl. 6, fig. 17; pl. 7, fig. 24). The function of the peculiar ladder-like structure is not known. On the ventral wall of the esophageal process is a heavy tuft of long cilia (pl. 6, fig. 17; pl. 7, fig. 24). They probably aid the cilia of the posterior part of the penniculus in directing the course of food and in forming food vacuoles. Only rarely have food particles, such as spores of *B. subtilus,* been found in this process, and they might have deposited there after the killing and fixing process. Apparently the cilia keep it free from foreign material.

Proceeding posteriorly from the dorsal wall of the cytopharynx at its junction with the esophagus are five or more heavy fibrils, and these terminate near the posterior end of the esophageal process in five large granules which are connected in a chain. These fibrils are neither tense nor very lax in stained specimens, but their condition in the living animal is not known. Because of their position they are called paraesophageal fibrils (fig. A, *1, paraeso. fib.,* pl. 1, fig. 1; pl. 3, fig. 3; pl. 6, fig. 21; pl. 7, fig. 28). They pass over the dorsal opening of the esophagus, and food material collects as it reaches these fibrils, so it is probable that they too aid in the concentration of food particles into vacuoles. Spores of *B. subtilus* were frequently found among these fibrils (pl. 2, fig. 1).

The chain of five granules in which the paraesophageal fibrils end, posteriorly, is usually connected only on the left side to the ladder-like terminal portion of the esophageal process. Since both ends sometimes appear connected, and in a few cases neither end is attached, their exact relation in the living animal is uncertain. This structure has, nevertheless, been called the posterior neuromotor chain (fig. A, *1, post. neur. ch.;* pl. 2, fig. 1; pl. 3, fig. 3; pl. 4, fig. 10; pl. 6, fig. 21; pl. 7, fig. 28), for from it pass three, and occasionally four, heavy fibrils toward the posterior end of the animal, which indicates some function other than merely that of anchoring the paraesophageal fibrils. It may be that this is the structure Gelei (1925) observed in *P. nephridiatum,* and called the "hinteres Nervenzentrum," but it corresponds more closely in position to his "terminal Protoplasmaflecken."

In addition to the three fibrils from the posterior neuromotor chain, from eight to a dozen fibrils pass posteriorly from the right wall of the cytopharynx toward the caudal end of the body. Usually six to eight of these can be traced beyond the esophageal process, so altogether about ten fibrils pass back, to be lost as they approach the posterior end of the animal. Their distal ends are unattached in stained preparations, and the fibrils are quite lax. These are the postesophageal fibrils (fig. A, *1, posteso. fib.;* pl. 2, fig. 1; pl. 3, figs. 2–4; pl. 4, fig. 10; pl. 6, figs. 17, 20, 21; pl. 7, figs. 24, 27, 28). These fibrils comprise the "Schlundfadenapparat of Bozler (1924), and are the "Stomodaealfasern" of Gelei (1925). Neither of these terms is especially suitable for the structure it is intended to designate. The writer accepts Bozler's explanation that the fibrils assist in the formation of food vacuoles, and that they direct the course of the vacuoles after they are formed. He does not believe them to be rigid, however, for frequently in stained animals they are wrapped almost around the food vacuoles. In the living animal they can be observed only a short way beyond the gullet, but certainly are not all in one plane, and are capable of some movement. All morphological evidence points to a lax condition of these fibrils, with their distal ends free.

DISCUSSION

On the basis of the descriptions of the silverline and neuromotor systems already given, the two systems may now be compared, in an effort to determine to what extent they share the same structures, and in what respects they differ.

Obviously, the first portion of the silverline system, designated by Klein as an "indirectly connected" conductile system, coincides very closely with the pellicular pattern described by many previous investigators, and briefly discussed by the author in introducing the neuromotor system. The silverlines in the hexagonal pattern mark only the ridges which bound the pellicular polygons. There is no fibril passing immediately beneath the pellicle which can be responsible for the effect produced, but the pellicle is slightly thicker at the top of these elevations than it is elsewhere. This, together with the tendency of the silver to collect to a greater degree on prominent surfaces, may be sufficient to explain the presence of the superficial silver lines. The writer has observed dried rotifers, eggs of nematode worms, bits of filamentous algae, and pieces of debris showing similar deposits on ridges and points, either natural or caused by shrinkage accompanying dehydration. The fact, previously suggested, that only in well dried specimens do the pellicular elevations collect the silver, indicates not a distinct structure with a chemical composition different from that of the surrounding protoplasm, but the operation of some physical principle, emphasized by drying. It will be remembered that with wet silver methods the pellicular part of the system is almost never demonstrated, and it is only natural to assume that with drying the elevations are the first to lose their film of water, and are the least likely to be protected by a film of salts left as the water evaporates. If the pellicle itself had a natural affinity for the silver, a more nearly uniform coloring would obviously result, so the interaction of the above factors, and possibly others also, must be responsible for this misleading set of lines.

The evidence for assuming that the lines of the "indirectly connected" conductile system are double is, as far as the writer can determine, derived almost entirely from a study of these lines at the corners of the polygons. The elevations in the pellicle are of appreciable width, and are necessarily even wider at points where three such elevations, each approximately 120 degrees apart, intersect smoothly. Often the centers of the intersections become slightly depressed as the animal dries. Consequently the most prominent ridges are not always the tops of the elevations, but frequently are the edges produced where these tops turn downward to form the sides of the dimples. It follows, therefore, that the three corners bounding the actual intersection of the pellicular ridges are subject to more intense drying, less protection by deposited salts, and other influencing factors, than is the intersection itself. Occasionally double lines appear in the sides of the hexagons, especially when the precipitation of silver is very heavy (pl. 7, fig. 25), and many other irregularities may be present, but, considering the drastic treatment to which the animals are subjected, such results are not surprising. The trichocyst granules demonstrated by the silverline method are identical with those found by the use of other methods. When more than one granule appears in the position marking a trichocyst, only one is the actual point of attachment of the trichocyst thread, and the others can usually be found to be connected with "accessory" fibrils or other structures variable in occurrence. As suggested previously, the writer attributes these occasional dots to the tendency of silver to collect wherever two or more fibrils intersect.

The longitudinal body fibrils, basal granules, and the associated fibrils and granules of less frequent occurrence, constituting what Klein calls the "directly connected" conductile system, is the subpellicular system demonstrated by many other techniques and described above as the peripheral portion of the neuromotor system. With the drying and flattening of the animal, it is brought considerably nearer the surface than it normally lies, and is thus easily reached by the silver nitrate. The basal granules, when demonstrated by any staining method, do not lie exactly in the centers of the hexagons, nor do the longitudinal body fibrils intersect them diametrically, but these discrepancies are more pronounced in silver preparations where much of the silver is coating rather than actually impregnating the structures. Accessory granules are undoubtedly just as numerous in specimens stained with iron-haematoxylin as in those treated with silver nitrate, but are extremely fine and escape attention, or cannot be seen at all, not having their diameter increased by precipitation, or possibly having only a slight affinity for this type of stain.

As pointed out by Gelei and Horváth (1931), one of the faults of Klein's method is its failure to penetrate the organisms. As a result, none of the pharyngeal structures are demonstrated, and consequently no correlation is possible. The method of Gelei and Horváth is therefore far superior to Klein's for staining fibrillar structures, since the greater share of the system lies in the pharyngeal region; and this method is recommended as the best simple technique for giving evidence of the pharyngo-esophageal network and penniculus, especially if time is limited. Even this wet silver method, which has so far proved satisfactory only for whole mounts, fails entirely to demonstrate many of the parts, and the rest of the pharyngeal parts only clearly enough for a superficial study, unless their nature is already clearly understood. The neuromotorium and endoplasmic fibrils have never been found in specimens prepared by this method, and the paraesophageal and postesophageal fibrils and the posterior neuromotor chain were identified with difficulty, but even this is, in the writer's opinion, commendable in whole mounts. Preparations lose their contrast rapidly if exposed to the light, and are therefore not particularly suitable for extremely detailed studies which may last over considerable periods of time.

The entire system described as neuromotor in an earlier part of this paper is an intricate one, not to be observed at a glance, but requiring great patience even in choice sections. Sagittal sections including only half of the cytopharynx and gullet are the easiest to interpret (pl. 1, fig. 1), but they comprise only a minute fraction of all the sections obtained in securing them.

The function assigned to the neuromotor system in *Paramecium* rests solely on morphological evidence, but the concept of conducting elements in Protozoa must not be the same as that obtained from a study of metazoan animals. Obviously, much of the above system might be suspected of furnishing tenacity to the surface of the body and the pharynx, and providing an anchorage for the cilia and various fibrils. Observations of crushed animals, both living and fixed, indicate that this is true to a certain degree, for the fibrils sometimes persist after other parts have been disrupted. There seems to be no evidence whatsoever that the fibrils could be myonemes, for they resemble those found in other ciliates neither in structure nor in their position relative to other parts. For similar reasons their function as supporting structures in the manner in which trichites yield rigidity to an organism is equally improbable. The pellicle with its peculiar relief undoubtedly acts in this capacity. The suggestion of Parker (1929) that fibrils may serve a "metabolic" function, presumably referring especially to nutrition or the distribution and utilization of food within a cell, is plausible in the case of neurons, whose processes may reach a length of nearly a meter; but to attribute such a function to so complex a network as is found in *Paramecium,* scarcely a fifth of a millimeter in length, is absurd. The endoplasmic fibrils might easily be conceived to function in correlating the feeding movements of the oral cilia with the condition of the endoplasm, in respect of food content, metabolic exchange, and other factors, but certainly this need not involve each cilium of the entire body.

On the basis of such considerations as the above, in addition to those previously mentioned in connection with the work of Sharp, a correlating function is not only "strongly indicated" for this system of fibrils, but is almost to be accepted without choice. This in no way excludes the possibility that the system may serve incidentally in yielding tenacity as well, and probably it may serve in still other ways, as yet not detected; for surprising as it is to find such an apparently simple organism to be so complex, it is inconceivable to the writer that each small part should have attained a degree of specialization comparable to that encountered in higher animals.

In conclusion, the "silverline" system, which has been given this name merely because of its demonstration by one particular technique, is composed not solely of conductile elements, but confuses parts of at least two and possibly three quite different aggregations of structures, namely, the pellicle, the trichocysts, and the peripheral portion of the neuromotor system. In addition, it fails completely to demonstrate the great pharyngeal complex, which is at least in part conductile. For these reasons, and because the term "neuromotor system" is descriptive of the function of the structure, and consequently of real biological rather than merely technical significance, as opposed to the superficial and unreal homogeneity back of the name "silverline," the use of the term "neuromotor system" is justified when referring to an apparatus in Protozoa which serves a correlating and conductile function. Since the final test of the function of this complex rests on experimental evidence, which is at present not available in the case of *Paramecium,* some investigators prefer to use the term "fibrillar system" to designate it. If the term "neuromotor" is understood in the broad sense which its compound origin indicates, the two terms designate, in the writer's interpretation, exactly the same system in *Paramecium.* The word "neuromotor" is, however, of biological connotation, whereas the term "fibrillar" is purely descriptive of individual parts, and without implication of integration, which is an essential part of the neuromotor concept.

SUMMARY

1. A comparative study has been made of the silverline system as demonstrated particularly by Klein's technique, and of the neuromotor system as demonstrated by the use of iron-haematoxylin and Mallory's stain.

2. The silverline system was found to be, as Klein had described it, composed of two rather distinct parts. One of these, Klein's "indirectly connected" conductile system, is composed largely of closely set polygons, chiefly hexagons, and the trichocyst granules which lie in the centers of the anterior and posterior sides of the polygons. The other portion consists chiefly of the basal granules, which are located at or near the centers of the polygons of the first part, and the longitudinal body fibrils, which connect the basal granules in the same longitudinal row of polygons. "Accessory" granules and fibrils also comprise a small part of this second portion.

3. The neuromotor system has been studied, and it too is composed of two general divisions. One of these is subpellicular, and consists of the basal granules and the longitudinal body fibrils, with certain other structures such as the suture lines, the lines about the cytopyge, and a curious set of radial fibrils which originate as longitudinal fibrils in the cytopharynx and esophagus and spread radially out from the oral opening over the body surface and terminate a short way from the cytostome. The second division is associated with the cytopharynx and gullet, and is composed of seven major parts, namely: (1) the pharyngo-esophageal network, (2) the neuromotorium, (3) the penniculus, (4) the esophageal process, (5) the paraesophageal fibrils, (6) the posterior neuromotor chain, and (7) postesophageal fibrils. All these parts have been figured and described, and their functions have been given, in so far as they could be determined.

4. The neuromotor system is conceived to have primarily a conductile function on the basis of the following considerations: (1) all parts of the system, with the possible exception of the penniculus, are connected (2) parts of this system supply every movable portion or structure of the body except the contractile vacuoles, (3) all the body parts, except possibly the cilia of the penniculus, are coordinated, (4) the structure, position, and manner of attachment of the fibrils do not indicate a supporting or contractile function, (5) the system and all its parts are comparable to those found in other ciliate Protozoa, and considered as neuromotor, and (6) no other conceivable function warrants a system so numerous in its elements and intricate in its construction, considering the facts already known about the nature and behavior of *Paramecium*. The fibrils are probably not extremely specialized, but serve only incidentally and secondarily, if at all, a strengthening function, by virtue of their tenacity. They do not add rigidity.

5. That portion of Klein's silverline system that is composed of polygons is purely pellicular, and not fibrillar in any sense. It colors heavily in well dried preparations because it is made up of pellicular ridges, which are the least protected by films of water and deposits of salts and offer better conditions for the deposit of colloidal silver than the smoother, less protected parts of the pellicle. The trichocysts are probably connected to the pellicle; hence, they too are not associated with the conductile system. The second or subpellicular portion of the silverline system is identical with the subpellicular fraction of the neuromotor system. Klein's method fails completely to demonstrate the most intricate part of the neuromotor complex, that associated with the pharyngeal region. Thus we see that the term "silverline" is only technical, and is morphologically confusing.

6. The wet silver method of Gelei and Horváth is superior to Klein's for a rapid study of the gross anatomy of the neuromotor system in *Paramecium.*

7. Because the silverline system confuses parts of three very different structures, the pellicle, trichocysts, and neuromotor complex; because it does not include more than a small part of the entire coordinating system; and because the name "silverline" is descriptive of the technique used to demonstrate the "system" rather than the structures themselves, the term "neuromotor system" is preferable when referring collectively to the conductile elements of ciliate Protozoa.

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EXPLANATION OF PLATES

All figures are of *Paramecium multimicronucleata*, and, unless indicated otherwise, are drawn with camera lucida from preparations fixed in Schaudinn's and stained with Heidenhain's haematoxylin.

PLATE 2

Fig. 1. Sagittal section 7.5 microns in thickness, showing the left half of the cytopharynx and esophagus and a portion of the posterior ventral half of the body. The circular and radial cytostomal fibrils are readily seen. Small portions of the longitudinal and circular pharyngeal fibrils may be seen, but they were largely obscured by the dense cilia of the penniculus. Larger portions of these fibrils are visible in the esophagus. The penniculus is very prominent, and the esophageal process, the paraesophageal fibrils, the posterior neuromotor chain, and the postesophageal fibrils stand out almost with diagrammatic clearness. Certain of the endoplasmic fibrils are visible, but the neuromotorium lies enough to the left to be obscured by the dorsal zone of cilia. The cilia of the pharyngeal region were not drawn in, for their presence would complicate the figure considerably.

A spore of *B. subtilus* is trapped in the paraesophageal fibrils. Two micronuclei, typical of the type found in *P. multimicronucleata*, are to be seen near the upper left of the figure.

The line drawing indicates the position of the section in the animal, and the scale below represents 30 microns. × 2250.

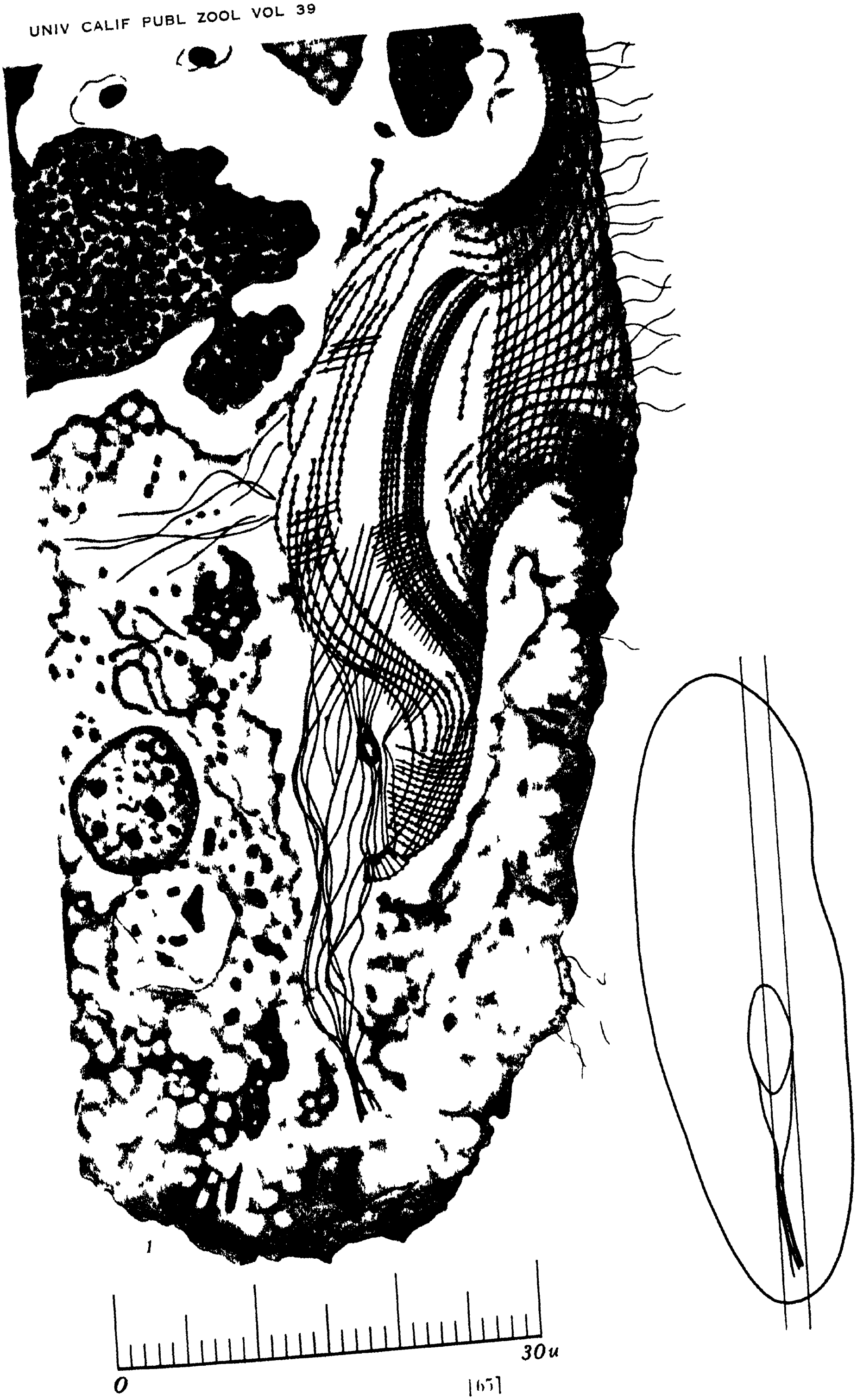

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PLATE 3

A series of five consecutive cross sections, each 7.5 microns in thickness and viewed from the posterior end.

The line drawing shows the positions of the sections in the animal. In the lower right is a scale of 20 microns. × 1250.

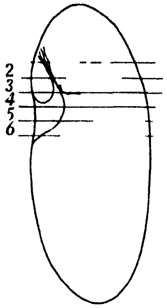
Fig. 2. Showing only the paraesophageal fibrils. The portion of the ectoplasm free from trichocysts marks the region of the cytopyge.

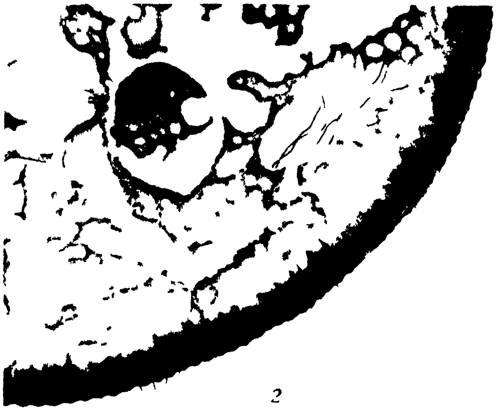
Fig. 3. Showing the posterior neuromotor chain, the paraesophageal and postesophageal fibrils, and portions of the circular and longitudinal esophageal fibrils. The cilia of the esophageal process, which are not shown, obscured many of the fibrils.

Fig. 4. Through the cytopharynx, showing the posterior margin of the cytostome with the circular and radial cytostomal fibrils, the penniculus, bits of the circular and longitudinal pharyngeal fibrils, the neuromotorium, and a few endoplasmic fibrils, and the proximal ends of the postesophageal fibrils, to the right. Cilia were not drawn.

Fig. 5. Showing the upper part of the cytopharynx, with circular and longitudinal pharyngeal fibrils, and their corresponding parts in the cytostome, the circular and radial cystomal fibrils. A few of the cilia at the upper end of the dorsal zone were drawn to indicate its position.

Fig. 6. Including the anterior margin of the cytostome, showing the circular and radial cytostomal fibrils.





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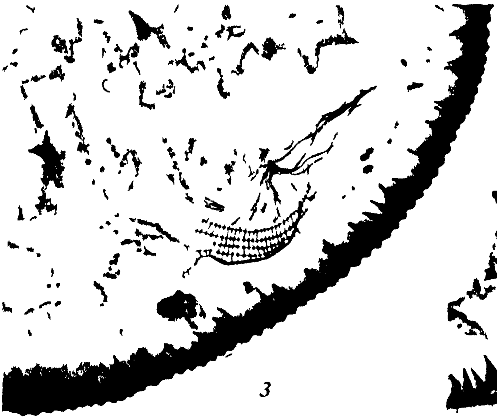

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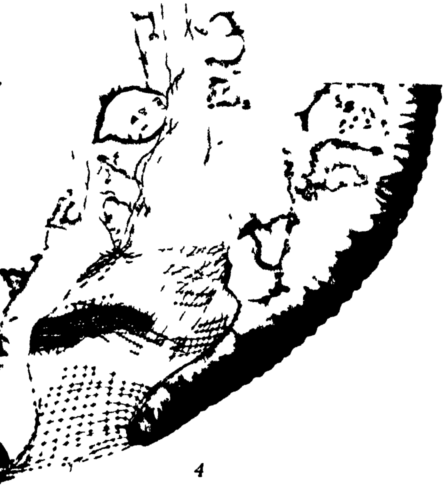

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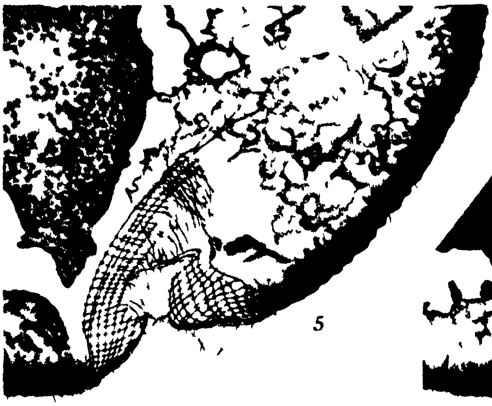

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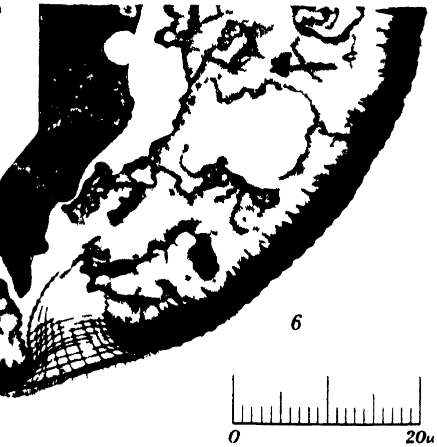

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PLATE 4

A series of four consecutive frontal sections, each 7.5 microns in thickness.

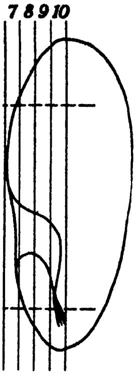
The line drawing shows the positions of the sections in the animal, and at the lower left is a scale of 30 microns. × 1375.

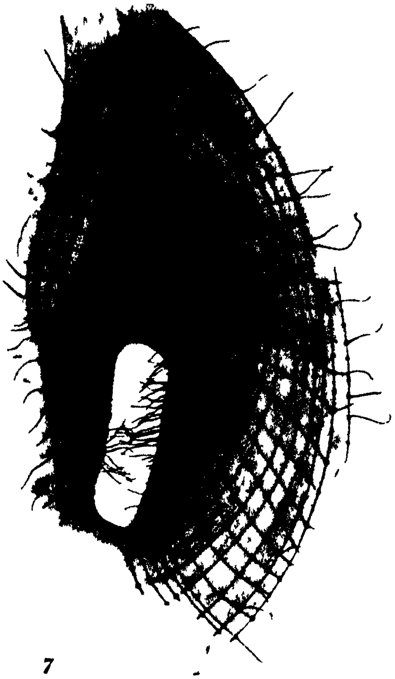
Fig. 7. Including the oral opening and a portion of the preoral suture, and showing in addition the circular and radial cytostomal fibrils and the cilia of the right and left walls of the cytostome. The cilia of the body surface have been omitted.

Fig. 8. Through the pharynx, showing portions of the circular and longitudinal pharyngeal fibrils and the cilia of the right and left walls of the cytopharynx. At the margin of the section the radial cytostomal fibrils are present, and the curving longitudinal body fibrils. Between this area and the cytopharynx are numerous dark bodies which are the trichocysts, sectioned in various ways.

Fig. 9. Showing the more dorsal and posterior part of the cytopharynx and the anterior ventral portion of the esophagus.

Fig. 10. Showing the dorsal wall of the cytopharynx, with longitudinal and circular pharyngeal fibrils, some endoplasmic fibrils, the esophagus and esophageal process, the posterior neuromotor chain, and the postesophageal fibrils. The paraesophageal fibrils and the neuromotorium lie too much to the dorsal side to be seen. Dense cilia of the dorsal zone, not shown in the figure, obscured underlying structures.

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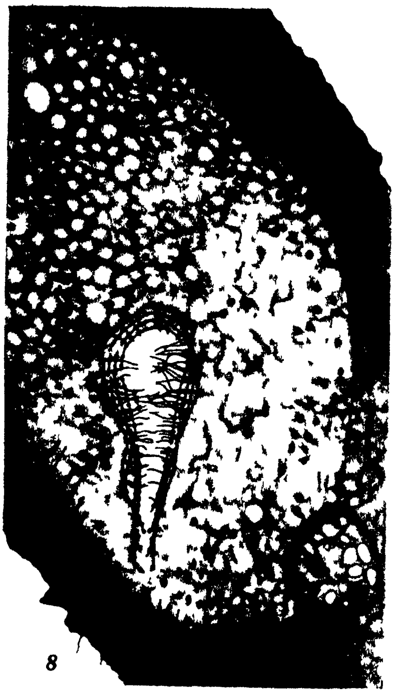
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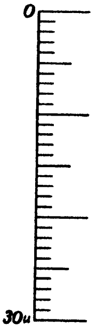

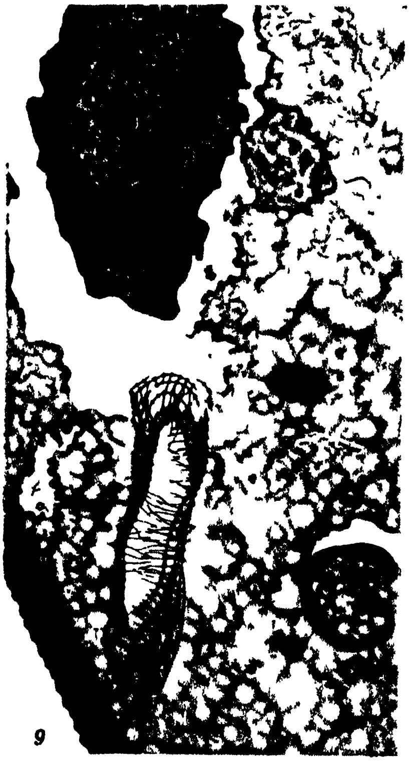
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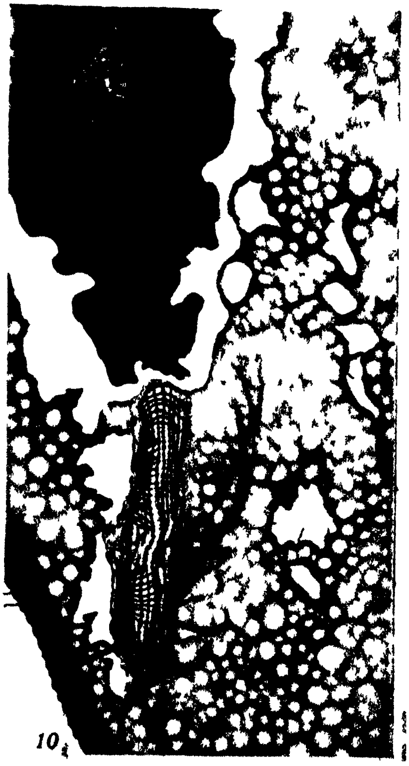
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PLATE 5

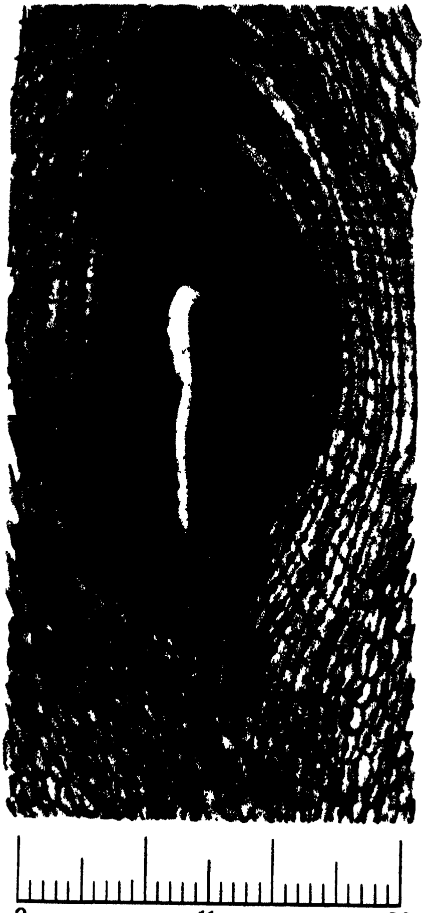
Fig. 11. The cytostomal region from a specimen prepared by Klein's method but not allowed to dry completely. Showing the longitudinal body fibrils, the circular cytostomal fibrils, the basal granules, and, more faintly, the pellicular ridges. A portion of both the preoral and the postoral suture is shown, and the outline of the cilia of the left cytostomal wall. Below is a scale of 30 microns. × 1700.

Fig. 12. Semidiagrammatic drawing of the pellicular structure and the basal granules and longitudinal body fibrils. Based on drawings and measurements taken with camera lucida from a tangential section of the anterior dorsal surface. × 7300.

Fig. 13. Same as above, but viewed in side elevation. Based on camera lucida drawings of longitudinal sections of anterior dorsal region. × 7300.

Fig. 14. Composite drawing based on figs. 12 and 13. × 7300.

Fig. 15. Showing internally discharged trichocysts in a specimen where they can be positively identified as such and not mistaken for ciliary rootlets or internal fibrils. Below is a scale of 30 microns. × 915.



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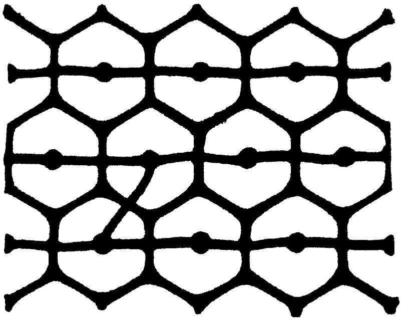

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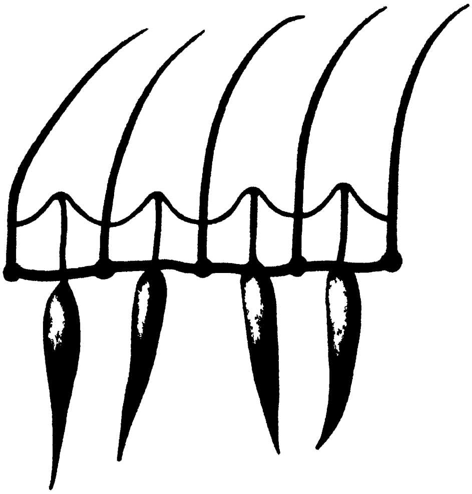

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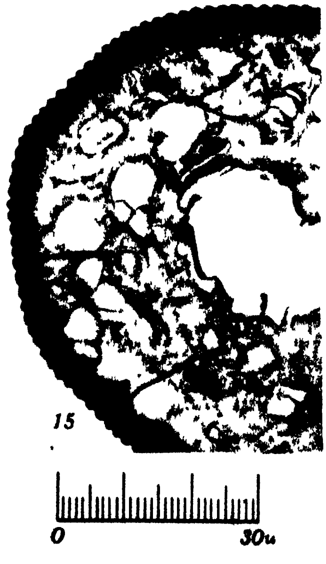

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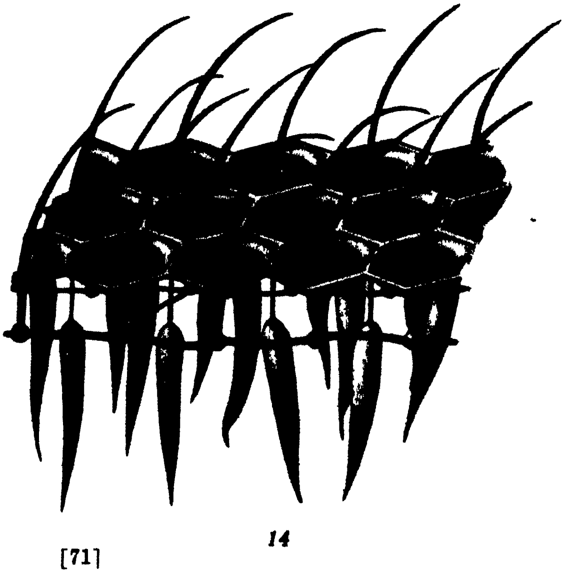

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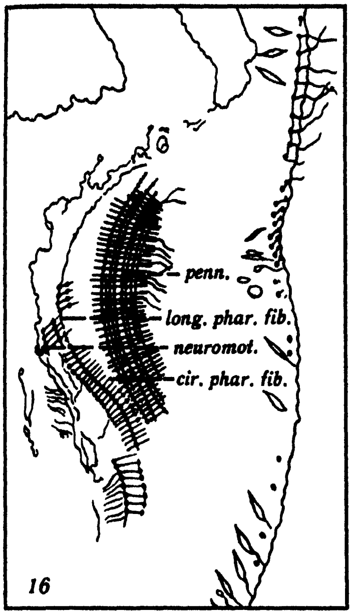
PLATE 6

Line drawings made with the aid of a camera lucida from the photomicrographs of plate 7. See explanations of figures of plate 7.

ABBREVIATIONS

penn.	**penniculus**
neuromot.	**neuromotorium**
long. phar. fib.	**longitudinal pharyngeal fibril**
cir. phar. fib.	**circular pharyngeal fibril**
dor. zone cil.	**dorsal zone of cilia**
cil. eso. proc.	**cilia of esophageal process**
eso. proc.	**esophageal process**
posteso. fib.	**postesophageal fibril**
bas. gr.	**basal granule**
acc. bas. gr.	**accessory basal granule**
long. bod. fib.	**longitudinal body fibril**
tric. gr.	**trichocyst granule**
pell. line	**pellicular line**
endopl. fib.	**endoplasmic fibril**
post. neur. ch.	**posterior neuromotor chain**
paraeso. fib.	**paraesophageal fibril**
cir. eso. fib.	**circular esophageal fibril**
***long. eso.* fib.**	**longitudinal esophageal fibril**



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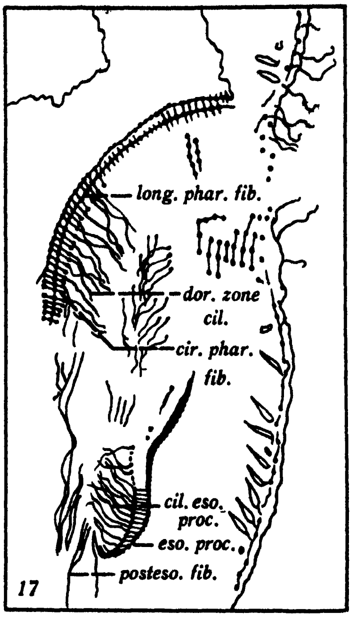

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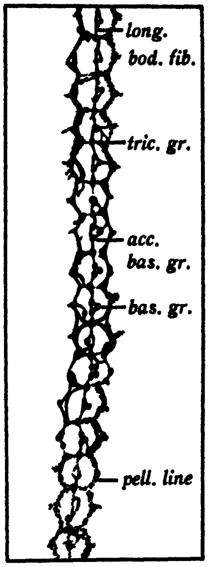

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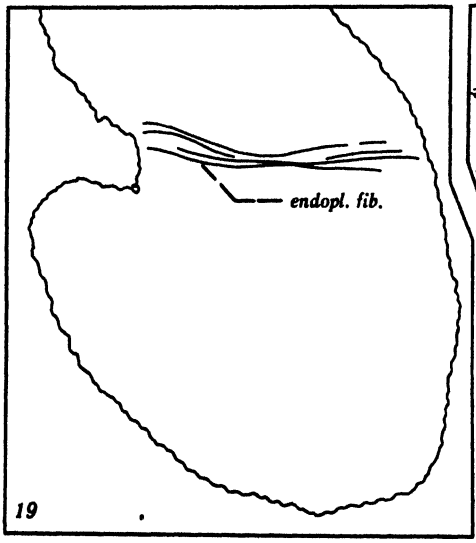

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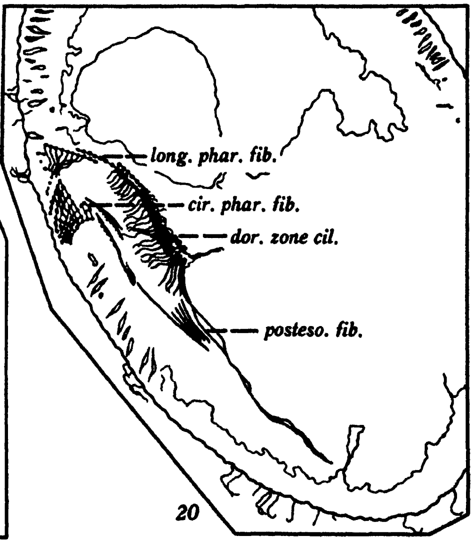

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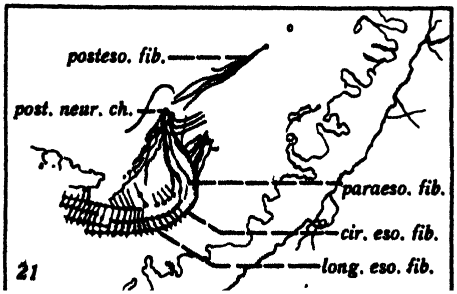

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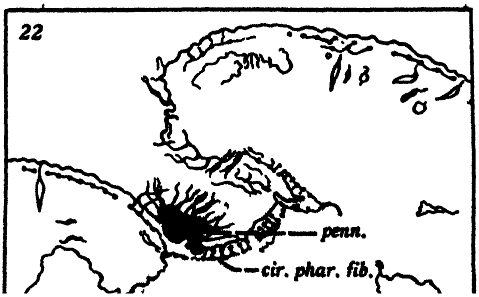

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PLATE 7

Photomicrographs taken by J. E. Gullberg

Fig. 23. Longitudinal section through cytopharynx and gullet, showing particularly the penniculus and the neuromotorium. The section was 7.5 microns in thickness. × 1050.

Fig. 24. Another level of the same section as the above, to show especially the longitudinal and circular pharyngeal fibrils and their intersections in the basal granules, the dorsal zone of cilia, the postesophageal fibrils, and the esophageal process.

Fig. 25. A portion of the dorsal surface of a specimen prepared by Klein's method and thoroughly dried on the surface before being treated with silver nitrate. Chosen to show especially the multiple nature of the granules near the centers of the polygons, i.e., the basal granules and accessory basal granules, and the apparent double nature of the pellicular lines in places. Accessory fibrils and other irregularities also occur. × 1850.

Fig. 26. Cross section, 7.5 microns in thickness, from an animal fixed in Zenker's with 2.5% acetic acid, and stained in Mallory's. Showing four prominent endoplasmic fibrils traversing the entire body. These lay at the extreme low focus, and the following section at high focus showed the neuromotorium, with which they undoubtedly had been attached. × 550.

Fig. 27. Oblique section, 7.5 microns in thickness, showing particularly the dorsal zone of cilia and the postesophageal fibrils. × 550.

Fig. 28. From cross section of 7.5 microns, showing especially the posterior neuro motor chain, somewhat coiled, and the paraesophageal and postesophageal fibrils. × 550.

Fig. 29. From cross section of 7.5 microns, showing an end view of the penniculus, also the circular pharyngeal fibril, and at the extreme left a portion of a radial cytostomal fibril. × 1050.

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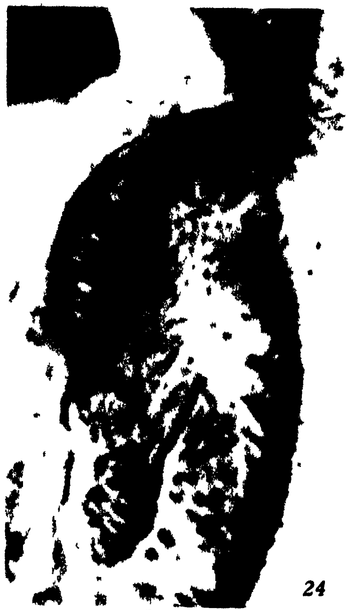

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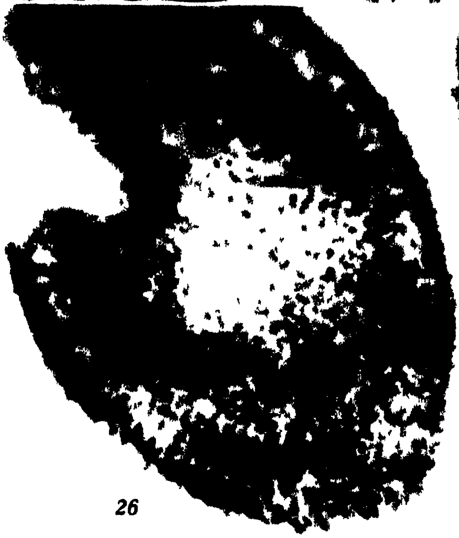

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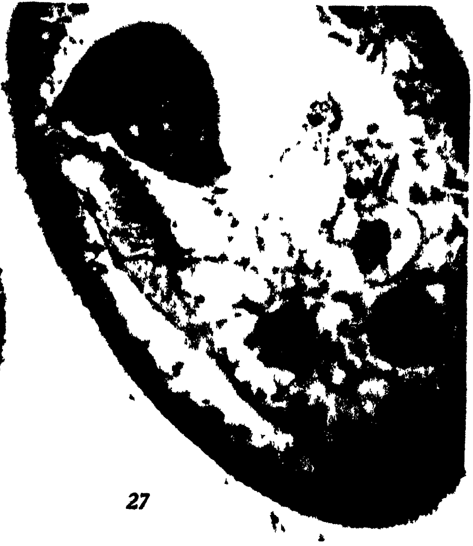

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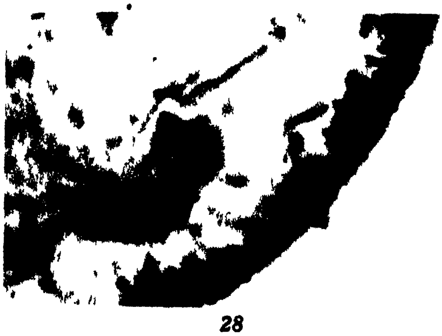

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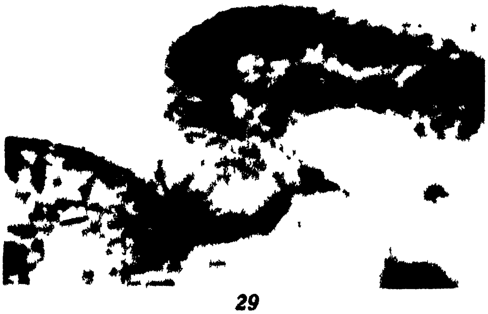

29

NEW SPECIES OF PROBOSCIDIELLA AND DEVESCOVINA FROM KALOTERMES OCCIDENTIS WALKER, A TERMITE OF LOWER CALIFORNIA

BY

WALTER W. LEWIS

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NEW SPECIES OF PROBOSCIDIELLA AND DEVESCOVINA FROM KALOTERMES OCCIDENTIS WALKER, A TERMITE OF LOWER CALIFORNIA

BY

WALTER W. LEWIS

INTRODUCTION

The family Oxymonadidae is characterized by the possession of a peculiar organelle of attachment, the rostellum. Of the two better known genera of the family, *Oxymonas* is uninucleate while *Proboscidiella* is primarily multinucleate. *Proboscidiella multinucleata,* the type of its genus, was described by Kofoid and Swezy (1926) and *P. kofoidi* by Kirby (1928). Another member of the genus, *P. occidentis* sp. nov., occurs in *Kalotermes occidentis* Walker. In the same termite is *Devescovina polyspira* sp. nov., a species of *Trichonympha,* and *Tricercomitus divergens* Kirby.

Advice and assistance in preparation of the paper have been given by Dr. Harold Kirby, Jr. and Dr. S. F. Light.

MATERIAL AND TECHNIQUE

The material for the study of two species of Protozoa from *Kalotermes occidentis* was loaned to the author by Dr. S. F. Light. The termites were collected in Lower California, Mexico, and the smears made and stained under Dr. Light's direction by Miss B. J. Andrew. The stains and fixatives used were Da Fano's cobalt nitrate silver as a combined fixative and stain, Delafield's haematoxylin after Bouin's fixative, and Heidenhain's iron alum haematoxylin, also after Bouin's.

Proboscidiella occidentis sp. nov.

MORPHOLOGY

There is a considerable variation in the shape and size of this species. The body is sometimes nearly spherical and at other times very much elongated, or it may be stretched partly around a piece of wood larger than itself, thereby conforming to the shape of the wood that it is trying to engulf. The usual body form is broadly ovoidal or pyriform, tapering towards the anterior end where the rostellum is attached. This organelle was termed the proboscis by Kofoid and Swezy (1926*b*) but may better be termed the rostellum. For the correction of nomenclature see Kirby (1928).

The average size of one hundred individuals was 36.2 microns wide by 63.5 microns long. The width varied from 11 to 80 microns, the length from 26 to 133 microns. The length was measured exclusive of the rostellum. The average length of the rostellum in one hundred individuals was 139.6 microns.

The body surface of *Proboscidiella occidentis* has a characteristic covering of bacteria. These are moderately long, usually curved, and often occur in great profusion scattered promiscuously over the surface, attached by one end to the protozoan. They stain deeply in iron-haematoxylin. A few bacteria are sometimes found attached to the rostellum (pl. 9, fig. 17). A thin layer of finely granular ectoplasm is usually present, varying in thickness from almost nothing to ten microns, and surrounded externally by a thin pellicle. The inner margin of the ectoplasm merges with the coarser endoplasm, which is filled with alveoli, and with certain slightly staining spherules always enclosed in vacuoles (pl. 8, fig. 12; pl. 9, figs. 16 and 19). These endoplasmic spherules seem to have a finely granular structure. The comparable yellow-brown spherules in *P. kofoidi* Kirby were believed to be a reserve food supply. There are still other structures, not figured, which vary in diameter from one-half the nuclear diameter to that of a large nucleus and are of loose construction, resembling nuclei without karyosomes. These peculiar structures appear in only a few individuals, but when present they often occur in large numbers.

In the endoplasm occur both large and small pieces of wood enclosed in vacuoles. Some show by their more rounded form the result of action of the digestive enzymes of the protozoan. The wood particles may be so large that the internal structures of the animal are pushed quite out of position. The engulfing of the wood evidently occurs by a pseudopodial action of the cytoplasm, reported by Kofoid and Swezy (1926*b*), and substantiated by the writer's observation of certain individuals fixed in the process of feeding.

As to cyst formation in *Proboscidiella,* the writer is much in doubt, although some evidence was presented by Kofoid and Swezy (1926*b*). Individuals with a conspicuous ectoplasm and no rostellum, resembling their figure of an apparent cyst, were seen in the writer's material. But individuals with conspicuous ectoplasm, rostellum, abundance of ingested wood, and elongated body have the appearance of being in an active state. Others without a rostellum show no conspicuous ectoplasm (pl. 8, fig. 12).

In the cytoplasm are certain tubular structures termed "vestigial sleeves" in *P. multinucleata* by Kofoid and Swezy (1926*b*), in reference to the similarity of appearance to the so-called sleeve of *Oxymonas.* The tubes appear to be of organic origin, many of them staining with iron-haematoxylin. They are found in a few Protozoa and in the intestinal contents of the termite. From one to five of them have been found in individual *Proboscidiella* (pl. 8, fig. 4). When found in the intestinal contents of the termite they are never stained, whereas when found in the Protozoa they may be unstained or they may stain in any degree to a very dark color. This staining reaction is relatively independent of the staining reaction of the protozoan host. One of these tubes has been found in *Devescovina polyspira,* in which it was of the characteristic yellow color as it appears in the intestinal contents of the termite and in some of the Protozoa. The tubes are not enclosed in vacuoles when in the cytoplasm. Outside of the flagellates several have been found of considerable length (63 microns) (pl. 8, fig. 6), and divided into as many as seven segments, which apparently break off into small sections, thus being more easily engulfed by the flagellates.

The conclusion is that the tubes are probably of plant origin, for they resemble tracheal tubes. The action of the digestive enzymes of the protozoan on the tubes have probably made them more stainable.

The nucleus in this species is usually in the anterior portion of the individual closely applied to the axostyle along one surface, which is the only apparent connection to the neuromotor system. The axostyle is usually peripheral to the nucleus (pl. 8, fig. 3). The number of nuclei in individuals of this species varies from one to seventeen, *P. occidentis* being the only species in this genus so far described with abundant uninucleate stages. The nuclei averaged in size, in fifty-seven individuals, 5.15 microns wide (3.8 to 8.8 microns) by 6.89 microns long (4.0 to 9.7 microns). In general the number of nuclei is in proportion to the size of the body. There may be an odd or even number of nuclei, and there may be a greater or smaller number of nuclei than of mastigonts (fig. A, 3–5).

The following tabulations give the relative numbers of nuclei per individual and the relative numbers of axostyles per individual:

Number of nuclei	1	2	3	4	5	6	7	8	9	10	11	12	13	17
Number of individuals	39	10	12	22	39	30	17	15	1	5	4	2	3	1
Number of axostyles	1	2	3	4	5	6	7	8	9	10	11	12	13	
Number of individuals	39	9	10	16	41	39	23	10	3	3	3	2	2	

These tabulations were made from approximately two hundred individuals of which sixteen (8 per cent) had a larger number of nuclei than axostyles and twenty (10 per cent) had a few extra axostyles. Eighteen per cent therefore had either extra nuclei or extra axostyles. It will also be noted that there is an abundance of uninucleate stages. Omitting the uninucleate stage, there is a steady increase of individuals with larger numbers of axostyles and nuclei up to five, and from there on the number of individuals with more mastigonts decreases to thirteen with the exceptional case of seventeen nuclei in an individual which contained only seven axostyles (fig. A, 4). Excluding the uninucleate stages, the average number of nuclei per individual is 5.5 in two hundred individuals.

The nucleus is bounded by a thin membrane. The chromatin material is usually in small, variously-shaped masses, sometimes apparently in a network yet not at any time definitely of a reticulate form. The nucleus ranges in size from 3.9 to 9.3 microns in diameter averaging about 6 microns in fifty-seven individuals. This is close to the size of the nucleus of the type species. The nucleus is usually spherical or ovoidal.

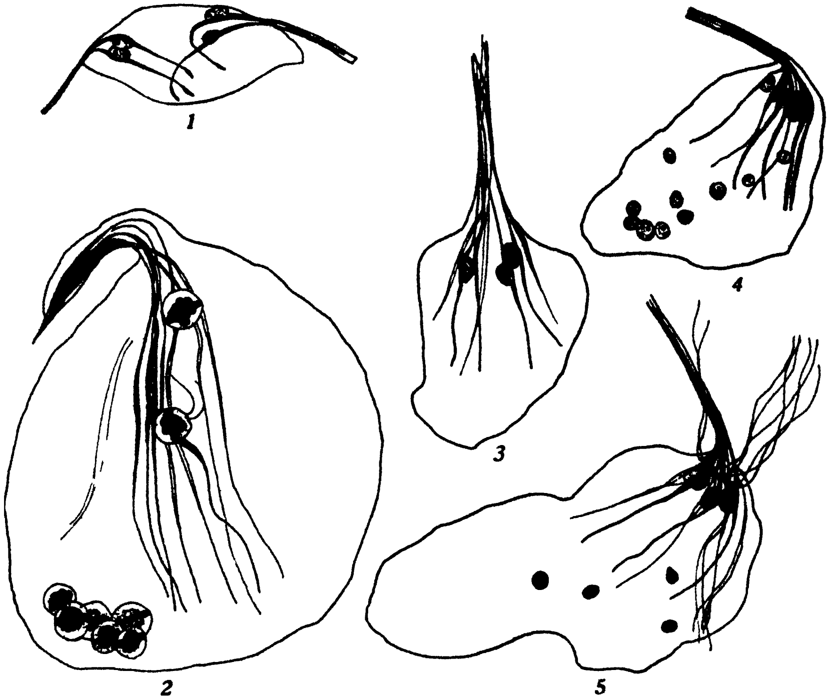

Fig. A. *Proboscidiella occidentis* sp. nov. *1.* Fission in which the nuclei have separated and two rostella are formed. *2.* Division form, migrated nuclei undergoing misosis; degenerate rostellum. *3.* Three nuclei and seven axostyles. *4.* Seventeen nuclei, seven axostyles. *5.* Ten nuclei, six axostyles. Magnification: *2*, × 740; *1, 3, 4, 5*, × 360.

Usually in the posterior part of each nucleus, is a clear space in which the karyosome is situated. The diameter of this space varies from 1.5 to 6 microns, averaging 3 microns. The diameter of the karyosome varies from 0.8 to 2.3 microns averaging 1.6 microns, which is about one-fourth the nuclear diameter. The karysome in this species is larger than in the type species and smaller than the karyosome in *P. kofoidi* Kirby. The clear space containing the karyosome often contains fibers radiating from the karyosome to the periphery. These may be only artifacts of fixation and staining; nevertheless, they are constant in all three species of this genus. Often there is a settling of chromatin material on the periphery of the clear space, in the form of small globules (pl. 9, fig. 13). A few cases have been found of the existence of an apparent lateral granule in the space.

The karyosome is of irregular outline and stains deeply in iron-haematoxylin. Occasionally a clear space is to be seen in its center, or there may be as many as four spaces in a single karyosome. This is shown clearly after Delafield's stain (pl. 2, fig. 16). The writer has no evidence that a central granule is present in it. Usually there is only one karyosome in each individual, but several individuals have been found in which a double karyosome or two definitely separated ones occurred (pl. 8, figs. 9 and 10).

The neuromotor system of this protozoan is different from those described in the other species in the genus. Cytoplasmic fibers, which were called by Kofoid and Swezy (1926*b*) "retractor fibers," are present in *P. occidentis,* extending longitudinally throughout the endoplasm. They are as long as the axostyles and have their origin in the rostellum (pl. 8, fig. 3). The neuromotor system of each mastigont is a double system in which two pairs of flagella, two blepharoplasts, rostellar fibers, a posterior rhizoplast, an interblepharoplast bar, an axostyle, and a nucleus make up the ensemble. The axostyle stains deeply with iron-haematoxylin and tapers very gradually at both ends to the thickest part which is in proximity to the nucleus. This tapering is distinct from the abrupt tapering of the axostyles of the other two species in the genus. Over the nucleus the axostyle is flat and ribbon-like, staining a lighter color because of its thinness. Its entire length is twisted and smoothly curved to the posterior end, which often has an arrow-shaped tip on it (pl. 9, figs. 15 and 19). The last third of the axostyle may be bent toward the pellicle of the body through which it may project. It often appears to be of a fibrillar nature. The anterior end projecting into the rostellum forms several strands of a rostellar fiber. The arrow-shaped end possibly drops off or shrinks to the usual pointed end.

The anterior flagella are about forty microns in length. The blepharoplasts are of nearly equal size, although one of each pair may be slightly larger than the other. From each blepharoplast a filament passes forward, possibly to join the axostylar filaments in the rostellum. One of the pair of blepharoplasts has another connection to the axostyle by a posteriorly projecting strand or rhizoplast which reaches the axostyle anterior to the nucleus (pl. 9, figs. 13 and 15). This posterior rhizoplast is not always discernible. The two blepharoplasts appear to be connected by a diagonal interblepharoplast bar of lightly staining material which connects the flagellar units beneath the axostyle (pl. 9, figs. 13, 15; pl. 8, fig. 11). The interblepharoplast bar is composed of a hyaline substance and is fairly wide (perhaps one-half a micron).

In the vicinity of the blepharoplasts, rhizoplast, and prenuclear portion of the axostyle in some individuals, there is a lightly stainable area which suggests a membrane (pl. 8, fig. 1; pl. 9, figs. 14 and 20).

Certain aggregations of granules have been found which are comparable to the similar granules found in *P. kofoidi* by Kirby (1928). These granules appear in the same place as the true parabasal body in some other flagellates. The granules (pl. 8, fig. 7), although sometimes scattered, are usually found in groups of two's or three's or in a curved line of five or six. There is no apparent connection between them. Their position is usually anterior to the nucleus where they lie parallel to the longitudinal axis of the body. They stain with iron-haematoxylin.

The width of the rostellum varies from 1 to 3.5 microns. Often there is a bulb about 4.4 microns wide at its distal end, or the tip may be truncate (pl. 8, figs. 2*a*, 2*b*). The length of the rostellum ranges up to 431 microns, averaging in one hundred individuals 139.6 microns, which is 2.2 times the length of the average individual. One protozoan with a body 40 by 60 microns had a rostellum 423 microns long, which is 7 times the length of its body. On plate 8, figure 12, is shown an individual without a rostellum. It is possible that the rostella instead of being retracted are detached, as a number of unattached rostella have been found in the intestinal contents. No examples of rostellar ends with a definite cup shape have been found. Many of the tips seem to be torn or frayed, as if the distal end had been broken off.

Two kinds of division have been observed in this species, binary fission and mitotic division. A few examples of simple binary fission have been found (fig. A, 1). Fission may be equal or unequal. Either way, the complete set of mastigonts remains intact, the division being merely plasmotomy. The first sign of the premitotic stage is the clumping together of the chromatin material and its drawing away from the nuclear membrane, leaving a large clear space surrounding the karyosome, which generally draws closer to the center of the nucleus. Next, the nucleus usually migrates to the posterior part of the body, where the process of division is completed. Only a few instances of the early stages were found. As the chromatin material clumps together a centrodesmose is formed extending from one side of the nucleus to the other (fig. A, 2; pl. 9, fig. 18). No paradesmose has been found.

During mitotic division the old neuromotor systems apparently are abandoned by the nuclei, and degenerate. No evidence of multiple fission has been seen on these slides.

Proboscidiella occidentis sp. nov.

Diagnosis.—Body about 36 by 64 microns; rostellum averaging a little over twice the length of the body; each mastigont including two blepharoplasts connected by a hyaline but stout interblepharoplast bar, each blepharoplast giving rise to a pair of flagella about two-thirds the body length; axostyle not differentiated into anterior axostylar process and axostyle proper, but continuing into rostellum; blepharoplasts connected to rostellar fibers which run parallel to the axostyle, and closely approximate if they do not join the anterior prolongation of the axostyle into the rostellum; nucleus with karyosome enclosed in a clear space; body usually containing characteristic granules as well as wood particles.

About 23 per cent of all the individuals are uninucleate forms. Excluding the uninucleate stages, the average number of mastigonts per individual is 5.5.

Long, hair-like micro-organisms attached by their ends cover the surface of the body.

Host.—Kalotermes occidentis Walker, Lower California, Mexico.

COMPARISON WITH OTHER SPECIES

The body sizes are approximately equal in the three species. The rostellum is recorded as longest in *P. multinucleata* and shortest in *P. kofoidi.* Uninucleate stages have been described in *P. multinucleata* and *P. occidentis.* In *P. kofoidi there* are from two to thirty-four nuclei per individual. The size of the nucleus in all three species is about the same. The karyosome of the type species is smallest, reaching only about one-fifth the diameter of the nucleus, while that of *P. kofoidi* is largest, averaging one-third the diameter of the nucleus. The karyosome of *P. occidentis* averages a little over one-fourth the diameter of the nucleus.

The tubular structures called ''vestigial sleeves'' have been found in the cytoplasm of all three species. The cytoplasmic fibers are said to be limited to the periphery in the type species, whereas they extend throughout the entire endoplasm in both *P. kofoidi* and *P. occidentis.* The anterior flagella in *P. multinucleata* are said to average about 18 microns in length, which is the shortest recorded in this genus. *P. kofoidi* has the longest flagella, averaging 60 microns, while in *P. occidentis* the flagella average 40 microns. In *P. multinucleata* only three flagella have been reported per mastigont; in the other species each mastigont has two pairs.

In all three named species of the genus there occur in each mastigont two blepharoplasts. These are connected by a stout blepharoplast bar in the type species, by an interblepharoplast filament in *P. kofoidi,* and by a stout but somewhat longer bar in *P. occidentis.* The arrangement of the rhizoplasts differs considerably in *P. multinucleata* and *P. occidentis.* There is no apparent rhizoplast connection between the blepharoplasts and the nucleus in *P. occidentis* as there is in *P. multinucleata.* The flagella of the type species are said not to be equal in thickness, but the flagella of *P. kofoidi* and *P. occidentis* are apparently of equal thickness.

Another distinct difference between this new species and the others is that the axostyle of *P. occidentis* tapers very gradually from its greatest width to its narrowest portions anteriorly and posteriorly with no such abrupt narrowing as occurs in both *P. multinucleata* and *P. kofoidi* at the juncture of the axostyle with the blepharoplast. Another difference is in the uniformity of staining that occurs in the axostyle of *P. occidentis* and does not occur in the staining of *P. kofoidi,* as seen on certain slides loaned to the writer by Dr.

Kirby. The latter species showed a lack of staining reaction of the axostylar prolongations that extend into the rostellum, even when slides were compared that were both stained and fixed with the same reagents.

The bacterial floras on the surface of all three species differ, as can be seen by comparing the drawings.

The geographical distribution of the three species of termites from which the three species of *Proboscidiella* came is very different. *P. multinucleata* occurs in a termite of the Philippine Islands, *Kalotermes* (*Planocryptotermes*) *nocens; P. kofoidi* in a Central American termite, *Kalotermes* (*Cryptotermes*) *dudleyi;* and *P. occidentis* is found in a Lower California termite that probably does not extend its range as far south as Central America.

Devescovina polyspira sp. nov.

MORPHOLOGY

Devescovina polyspira sp. nov. has a broadly ovoid body ranging in width from 9 to 44 microns, averaging 21.1, and in length from 24 to 94 microns, averaging 44.8 in 81 individuals. There are apparently two variants in size and shape in this species; one, a slender elongated type, the other ovoid, rotund, and somewhat larger. Definitely intergrading sizes show that they are both of the same species, although the larger one usually has the split parabasal body while the smaller has a simple parabasal body. The anterior end is often extended by a papilla.

Few adherent micro-organisms are to be found on this protozoan, and those few are usually in small clusters on the posterior or anterior end. There are no cuticular striations in this species.

The cytoplasm is alveolar and finely granular between the alveoli. All foreign inclusions are contained in vacuoles. The body is engorged with wood particles, and a few bacteria are to be found in the cytoplasm, always within vacuoles. Parasites were found in the cytoplasm of a few individuals (pl. 10, fig. 26).

An internal clear area extending from the posterior end of the nucleus to the body surface on one side of the nucleus, usually opposite the cresta or chromatic basal rod, has the appearance of a cytostome (pl. 9, fig. 21; pl. 10, figs. 31 and 32). There does not appear to be so complex a structure as the group of clear areas and internal membranes of *Metadevescovina debilis* Light (1926).

The single nucleus at the anterior end of the body, with a membrane which is irregular and wrinkled, is usually bluntly pointed at the anterior end and broadly rounded at the posterior end. The chromatin material in the resting stage is contracted from the nuclear membrane, leaving a clear zone. The chromatin material is densely staining in Heidenhain's iron-haematoxylin, Delafield's haematoxylin, and Da Fano's cobalt nitrate silver. The mass of nuclear chromatin is often lengthened transversely, with rough uneven boundaries, and with dimensions of about 3 by 5 microns. The dimensions of the region enclosed by the nuclear membrane reach 6 by 7 microns, but average 4 by 5 microns in average sized specimens. The densely staining eccentric karyosome which is about one micron in diameter, is enclosed in a clear space 1.5 microns in diameter. Usually there is only one karyosome, but several specimens have been found with two karyosomes. A posterior projection of densely staining nuclear material projects from the nuclear mass in the direction of the axostyle.

The neuromotor apparatus consists of blepharoplast, cresta or chromatic basal rod, trailing flagellum, three anterior flagella, parabasal apparatus, and axostyle. The blepharoplast is about one-half a micron in diameter and appears in some individuals to consist of two or three granules. The subtriangular, densely staining cresta is attached to the blepharoplast by a thread (pl. 10, fig. 31). It varies in length from one and a half to nearly three times the largest dimension of the nucleus. In an individual 25 by 43 microns, containing a nucleus 5 by 6 microns, the cresta was 15 microns long. The attached end or base of the triangle is flat and broad, tapering to a bluntly pointed rod at its distal end. The trailing flagellum remains so close to the cresta during the first part of its course, that often, when the flagellum is projected anteriorly, the cresta swings around and closely approximates its course, although probably it never protrudes through the pellicle of the protozoan. The trailing flagellum tapers to a fine point at both ends with a very long taper to a most delicate thread at the distal end. The entire length is from one to one and a half times the body length of the protozoan. The flagellum is not flat but round and whip-like.

Three delicate anterior flagella of nearly one and a half times the body length extend from the blepharoplast. They are of practically the same diameter throughout their entire length except for a slight taper in their distal portion. In many individuals the anterior flagella seem to be attached at their proximal ends to the protozoan for a distance of from 4 to 6 microns. This attachment of the anterior flagella to the body surface is similar to that described by Grassi (1917) for *Devescovina glabra*.

In plate 10, figure 24, is a figure which appears a bit puzzling as the microorganisms on the anterior end resemble what might easily be taken for another group of flagella. Several specimens have been found which present the same appearance, and almost all individuals appear to have certain radiating lines in a fan shape anterior to the nucleus. These lines appear to be as thick as the "basal granules" of *Metadevescovina debilis* Light (1926), which were the bases of the supposed tertiary flagella.

The hyaline axostyle, which is usually curved and fibrillar, flares at the anterior end to partially surround the nucleus. An enlargement at the posterior end as it projects from the body has a siderophile muff or ring about it. Posterior to the muff, the axostyle tapers to a point. The axostyle in this species is probably very flexible, for in the fixed specimens the axostyle is found in every degree of flexion, sometimes paralleling the rotund curve of the body wall. The anterior end of the axostyle is in contact with the blepharoplast.

There were prominent in some individuals certain darkly staining, enigmatic structures which resemble a comma in shape (pl. 10, fig. 24). One was attached by its blunt end to the blepharoplast and the other was free and separated by a space of only a few microns.

The split parabasal apparatus is the most distinctive characteristic of this species. The apparatus consists of a parabasal body with a parabasal thread which extends its full length along the inside margin and attaches to the blepharoplast. The parabasal body continues with the same diameter from its anterior to its posterior end. Distally the parabasal apparatus may occur unsplit or may be partially divided into as many as six or eight strands, each making from three-fourths to two and a half turns about the axostyle and nucleus and continuing with the same diameter to a blunt end. This resembles the parabasal apparatus in *Devescovina nova* Grassi (1917) except that instead of being extended posteriorly as far as Grassi's drawings show it to be, the parabasal apparatus of this species is more closely coiled around the anterior portion of the axostyle in a left spiral. In material prepared by Bouin's fixative and iron-haematoxylin, the parabasal body is hyaline, although the parabasal thread stains darkly enough to be seen. In Delafield's haematoxylin and Da Fano's stain the parabasal body appears vacuolated much the same as in the "vesicules chromophobes" of *Devescovina hilli* Duboscq and Grassé (1928). Usually one row of vesicles only is present, but other individuals have two rows, or the vesicles may be of smaller size and scattered in no definite order in the parabasal body (pl. 10, figs. 27–30).

Few division stages were found in the material at hand, but it is obvious that the type of division (pl. 10, figs. 25 and 26) is the same as that of the other species of *Devescovina.*

Devescovina polyspira sp. nov.

Diagnosis.—Dimensions of the broadly ovoid or pyriform body averaging 21 by 45 microns; oval nucleus with a clear zone between the central chromatin mass and the distinct nuclear membrane and an eccentric karyosome surrounded by a halo; often a cone of darkly staining material projecting posteriorly from the chromatin material of the nucleus toward the axostyle; three delicate anterior flagella; trailing flagellum slender, round, and tapering at both ends; trailing flagellum and anterior flagella one and a half times the body length; subtriangular cresta in length one and a half to three times

the diameter of the nucleus; fibrillar hyaline axostyle passing around one side of the nucleus, often projecting posteriorly in a tapering point, encompassed by a siderophile muff at the point of emergence from the body; parabasal body making three-fourths to two and a half laeotropic turns about the nucleus and axostyle, distally often split into as many as six or eight strands; connected to the blepharoplast by a peripheral parabasal thread which extends throughout its entire length; anterior clear area on one side of the nucleus clearly discernible, extending into the body at least as deeply as the posterior end of the nucleus.

Host.—Kalotermes occidentis Walker, Lower California, Mexico.

SUMMARY

1. *Proboscidiella occidentis* sp. nov. is described from the termite *Kalotermes occidentis* Walker, of Lower California, Mexico.

2. Tubular structures sometimes found in the cytoplasm are probably of plant origin, possibly tracheal tubes.

3. The average number of nuclei per individual is 5.5, but about 23 per cent of all individuals observed were uninucleate.

4. The two blepharoplasts in each mastigont are connected by a stout, hyaline interblepharoplast bar, and each blepharoplast gives origin to a pair of long flagella.

5. *Devescovina polyspira* sp. nov. is described from the same termite.

6. This species is unusual in that the parabasal is often split, dividing distally into as many as six or eight strands.

7. A fibrillar, hyaline axostyle with a terminal expansion and a siderophile muff at the distal end is present.

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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida, excepting figures 23 and 32 on plate 10. Abbreviations for methods of preparation: B., Bouin's fixative; H., Heidenhain's iron haematoxylin; D., Delafield's haematoxylin; and D.F., Da Fano's stain.

PLATE 8

Proboscidiella occidentis sp. nov.

Fig. 1. Uninucleate individual, showing flagella, axostyle, blepharoplasts, and rostellum. Note apparent membrane anterior to the nucleus. B.H. × 740.

Fig. 2. *a*. Bulbous end of rostellum. B.H. × 1385.
b. Truncate end of rostellum. B.H. × 1385.

Fig. 3. Showing close application of axostyles to nuclei; detached nucleus in posterior portion of body; cytoplasmic fibers; endoplasmic spherules. B.H. × 740.

Fig. 4. Tubular structures in the cytoplasm, five in number; large wood particle in posterior portion of individual. B.H. × 740.

Fig. 5. Tubular structures in cytoplasm, colored yellow, or unstained.

Fig. 6. Yellow-colored tube evidently of the same material as those shown in figures 5 and 6 but found free in the intestinal contents of the termite; note the segments or joints which would probably allow easy cleavage of parts about the same length as those found in the protozoan body. B.H. × 740.

Fig. 7. Showing groups of granules anterior to nuclei; flagella; wood particles. B.H. × 740.

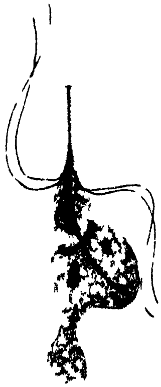
Fig. 8. Nucleus of a uninucleate individual, showing clumped chromatin. B.H. × 1385.

Fig. 9. Nucleus showing condensed chromatin and double karyosome. B.H. × 1385.

Fig. 10. Two karyosomes; massed chromatin; anterior part of axostyle. B.H. × 1385.

Fig. 11. Showing the detailed structure of the mastigonts, and arrow-shaped tips on the axostyles. D.F. × 740.

Fig. 12. Rostellum retracted or missing; note fibrillar anterior portion of axostyle; wood particles in cyptoplasm enclosed in vacuoles; flagella. B.H. × 740.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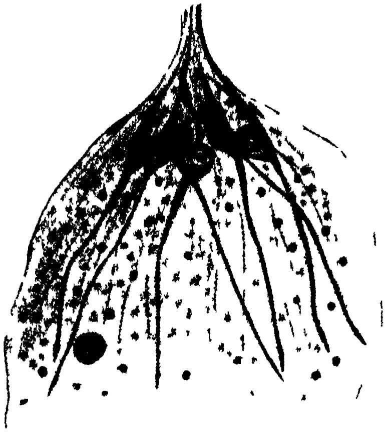
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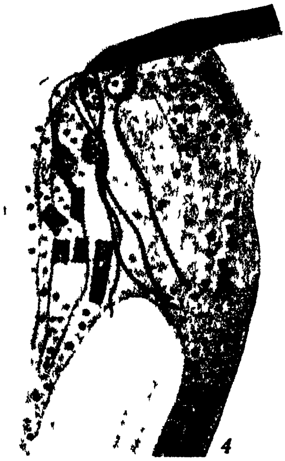
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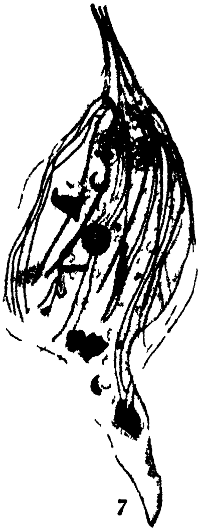
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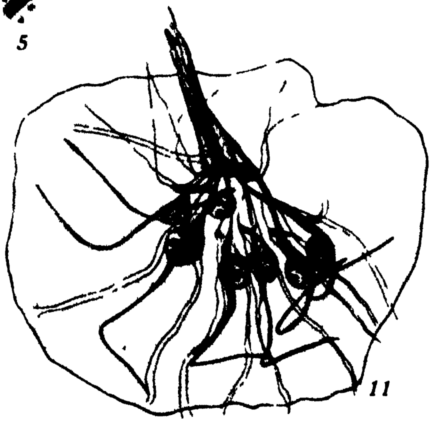
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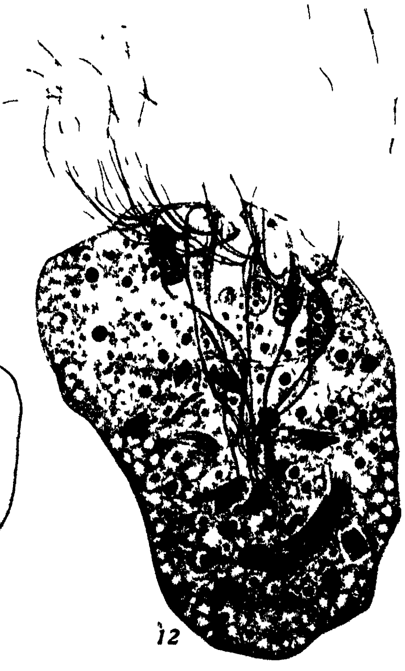
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PLATE 9

Figures 13–20. *Proboscidiella occidentis* sp. nov.

Fig. 13. Semidiagrammatic drawing of neuromotor system, with resting nucleus, in a uninucleate protozoan; nuclear granules grouped around halo, as frequently occurs. B.H.×1385.

Fig. 14. Apparent membrane anterior to the nucleus which is often found in one and two-nucleate flagellates. B.H. × 1385.

Fig. 15. Neuromotor system drawn from individual illustrated in pl. 1, fig. 11; showing rostellar filaments, rhizoplast, blepharoplasts, interblepharoplast bar, axostyle with arrow-shaped posterior end and anterior extension, and flagella. B.H. × 1385.

Fig. 16. Vesicles in karyosome; *Tricercomitus* attached to the rostellum; rostellum is 102 microns long in this specimen. B.D. × 740.

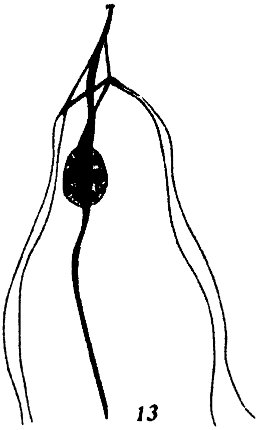
Fig. 17. Showing microorganisms on the surface of the body. These occur on nearly all individuals, but have been omitted in other drawings. B.H. × 740.

Fig. 18. Division form of nucleus, showing centrodesmose. B.H. × 1385.

Fig. 19. Ectoplasm, endoplasm, endoplasmic spherules in vacuoles; cytoplasmic fibers; elongated blepharoplasts; wood particles in cytoplasm; arrow-pointed tips on axostyles; radiating threads from karyosome to periphery of clear area. B.H. × 740.

Fig. 20. Uninucleate individual showing apparent membrane anterior to the nucleus. B.H. × 1385.

Fig. 21. *Devescovina polyspira* sp. nov. Entire individual; optical section through parabasal body, with surrounding clear space, showing the parabasal thread in cross section; fibrillar axostyle; bacteria (?) coiled in vacuole; wood particles; muff or ring on the end of the axostyle; three anterior and one trailing flagella; cresta or chromatic basal rod. B.H. × 1385.



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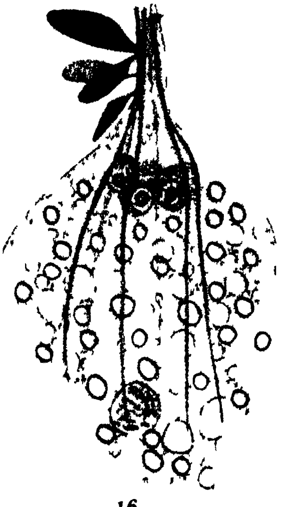

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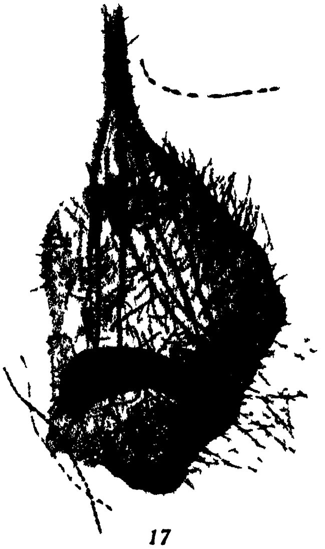

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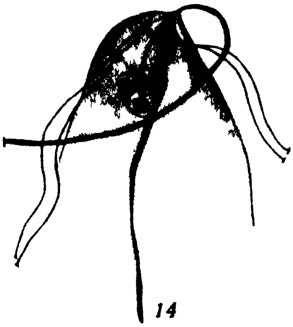

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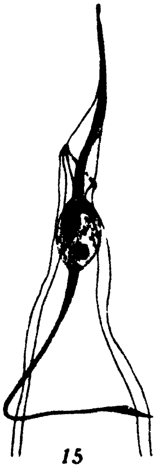

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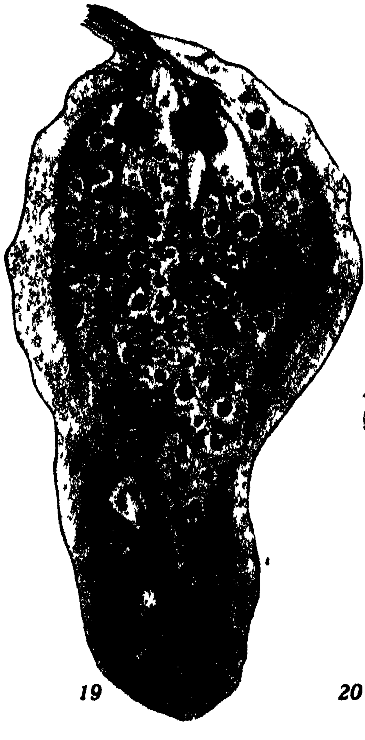

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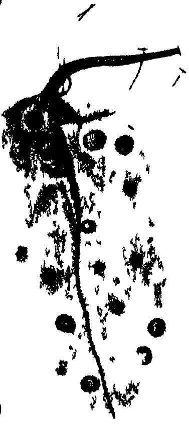

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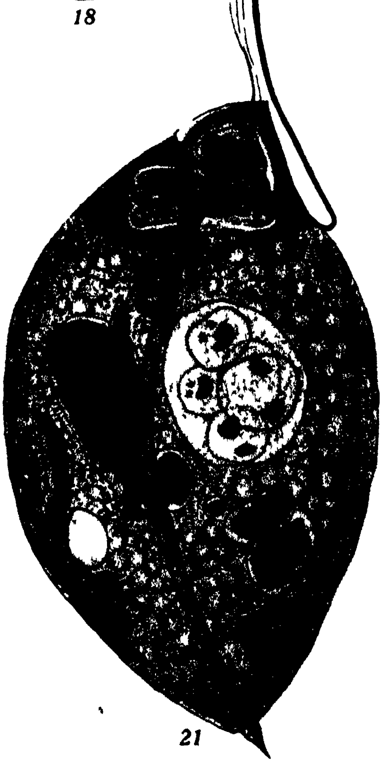

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PLATE 10

Devescovina polyspira sp. nov.

Fig. 22. Posterior projection of stained chromatin material in nucleus; cresta. B.H. × 1385.

Fig. 23. Transverse nucleus; karyosome surrounded by clear area. D.F.

Fig. 24. Group of adherent spirochaetes attached anterior to nucleus, simulating a group of flagella. B.H. × 1385.

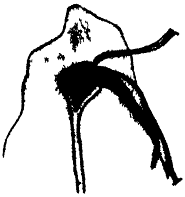
Fig. 25. Division form; fibrillar axostyle, enlarged; paradesmose; club-shaped parabasal bodies. D.F. × 1385

Fig. 26. Division form with split parabasal body; fibrillar axostyle; parasite in cytoplasm, common in this protozoan. D.F. × 1385.

Figs. 27–30. Parabasal bodies, showing vesicles and split posterior portions. The more complex parabasal apparatus, as indicated by splitting, occurs in flagellates of larger size. That containing the parabasal body of fig. 27 measured 17 by 48 microns; that of fig. 30, 28 by 88 microns. × 1385.

Fig. 31. Flagella, papilla, blepharoplast, cresta with clear area adjacent to it, parabasal apparatus barely visible. B.H. × 1385.

Fig. 32. Sketch showing the clear area curving around the nucleus; gentian violet stain used.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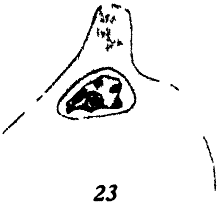
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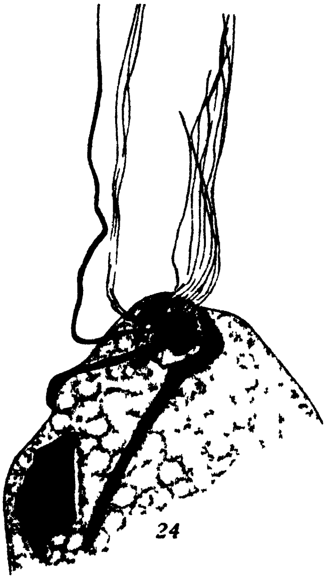
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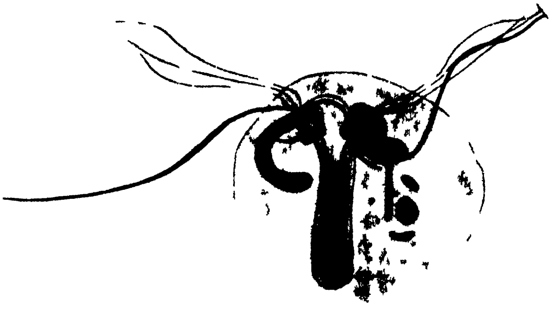
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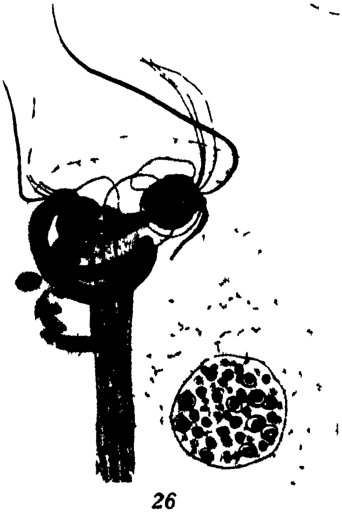
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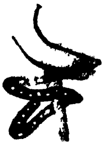
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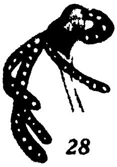
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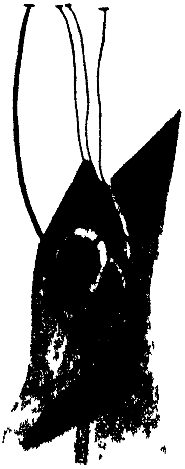
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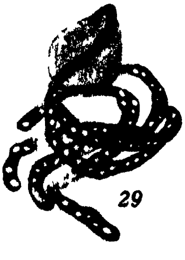
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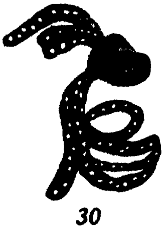
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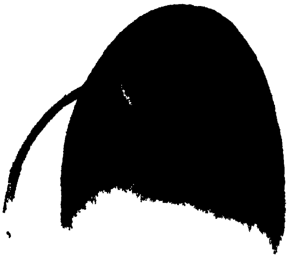
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A NEW BLIND ISOPOD, ASELLUS CALIFORNICUS, AND A REVISION OF THE SUBTERRANEAN ASELLIDS

BY

MILTON A. MILLER

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A NEW BLIND ISOPOD, ASELLUS CALIFORNICUS, AND A REVISION OF THE SUBTERRANEAN ASELLIDS

BY

MILTON A. MILLER

INTRODUCTION

The blind isopods here described possess the characters ascribed to *Caecidotea* Packard. After a comparative investigation of that supposed genus and the genus *Asellus* Geoffroy, however, I have concluded that *Caecidotea* is invalid for reasons to be given, and that the species grouped under that name should be placed under *Asellus*. Consequently the new species here described is placed under the latter genus.

The specimens were collected by Professor E. O. Essig from a renovated fresh-water well on the ranch of Mr. William Tuttle near Kelseyville, Lake County, California, in March 1931, and were given to Professor S. J. Holmes, who turned them over to me for examination. Mr. Tuttle later kindly furnished me with information regarding the circumstances of collection. As none of the females was carrying young, attempts were made by Professor Essig through letters to Mr. B. C. Barnard, County Agent, United States Department of Agriculture at Kelseyville, and by myself through a visit to the ranch in June, 1932, to secure more specimens, but we were not successful.

I am indebted to Dr. Holmes for permission to examine the material, and for helpful advice and criticism. Thanks are also due Professor Essig, Mr. Tuttle, and Mr. Barnard for the courtesies shown me.

Asellus californicus sp. nov.

Figures 1–14

Description.—All specimens taken, of about same size. Largest specimen (male) little more than five times longer than wide, 6.2 mm.: 1.2 mm. Color, in alcohol, creamy white. Body loosely articulated, of practically uniform width. Surface smooth; margins of segments fringed with hair.

Head longer and slightly narrower than first thoracic segment, three-fourths as long as wide. Front widely notched for insertion of antennae. Postmandibular lobes small but distinct. Eyes absent.

Flagellum of first antenna with eight segments; tip of flagellum extending to distal end of fourth segment of second antenna. Basal articles of peduncle inserted close together, but broad and expanded distally. Second article equal to first save about one-half narrower. Third article about three-fifths as long as second. Each article with a few setae mostly concentrated at distal end. Apices of segments three to eight of flagellum bearing a club-shaped seta (olfactory?) in addition to one or two normal setae.

Second pair of antennae about three-fourths as long as body, with delicate, multiarticulate flagella. First three articles of flagellum short, second slightly longer than first and third, and each broader distally than proximally. Fourth article about length of first three, but narrower. Fifth article one-fifth longer than fourth, and slightly narrower. Each article of the peduncle bears a few setae, longer and more concentrated at apex. Flagellum with about forty articles, each with one or two setae.

Upper lip small, rounded, densely fringed with hairs.

Mandible (fig. 8) with two sets of teeth at the apex, outer with three, inner with four teeth. On margin below inner series of teeth are five plumose setae. Molar expansion prominent with fine triturating surface. Palp with three articles.

Lower lip (fig. 10) consisting of two roundly triangular lobes, distal and inner margins of which are densely hairy.

First maxilla (fig. 9) with two terminal laminae, inner shorter and one-half narrower than outer and furnished at tip with five plumose setae. Outer lamina with about twelve apical spines, innermost half of which are stoutly and irregularly pectinate, while the outer become longer and smooth.

Second maxilla (fig. 11) with two branches, inner lamina much shorter than outer which consists of two subequal laminae. Distal margin of inner lamina sinuate, receding toward mid-line, and fringed with setae. Distal margins of the two laminae of outer branch with microscopically pectinate setae, varying from long outer to short inner setae.

Maxilliped (fig. 12) operculate, with large palp of five articles. Apex of inner plate, outer and distal margin of epipodite, and margins of last four segments of palp heavily setose. Basipodite rounded laterally, nude. No sexual dimorphism observed.

Segments of thorax loosely articulated, with lateral margins parallel but not contiguous. Segments vary but slightly in length according to the formula $1>2=7>3=6>4>5$. Epimera visible dorsally, antero-lateral on first to fourth segment, becoming posteriorly situated on lateral margin on last three.

Legs slender, uni-unguiculate; first pair (fig. 13) subchelate and shorter than the rest. Dactylus curved with two short spines on its concave margin, and several setae on its convex margin. Palmar margin of propodus with three stout spines and several setae. Carpus and merus small and triangular, the latter with two spines on its apex. Ischium with one small spine in middle of its inner margin. Of the remaining six pairs of legs in both sexes, the fourth pair is shortest, but little longer than first pair. Each of the other pairs are successively longer than preceding pair; seventh pair (fig. 14) about one and three-fourths longer than first. Apex of merus of each pair of legs is strongly produced and armed with two to five long spines. Distal outer margin of propodus of last pairs of legs with a single plumose seta. Females with small ovate oöstegites at bases of first four legs, those of last two pairs large enough to slightly overlap at mid-line.

Abdomen with first and second segments small but visible dorsally. Pleotelson one-fifth longer than wide, rounded, and slightly truncate posteriorly. A transverse line, not extending to lateral margin, near anterior end of pleotelson probably indicates coalesced third segment.

Uropoda slender, one-third longer than abdomen. Peduncle about as long as inner ramus, and twice as long as outer ramus. Rami tipped with long setae; peduncle and rami spinulose.

First pleopod of male (fig. 1) consisting of a short, squarish, basal plate, and a broad, ovate, terminal ramus. Distal lateral margin of ramus slightly produced into a rounded lobe. Lateral and distal margins of ramus fringed with setae. First pleopod of female (fig. 7) reduced, consisting of a small triangular plate.

Remaining pleopods in both sexes biramous, showing an interesting serial modification of parts. Second pleopoda absent in female, while in male they are modified into a sexual appendage. Third, fourth, and fifth pairs essentially similar in both sexes, showing a progressive reduction of the exopodite.

Sympod of second pleopod of male (fig. 2) consisting of a squarish, flat plate, about twice as large as that of first pleopod. Exopod and endopod biarticulate, attached to distal margin of sympod. Basal article of exopod small with short spine on its outer distal margin. Distal segment longer and wider with five or six setae on its outer margin. Endopod modified into a copulatory organ. Basal segment small with a curved process extending toward inner margin of sympod. Distal segment bulbous at the base, tapering into a long cylindrical process which curves outward over exopod. A fine needle-like stylet originates in bulbous part, runs up through a sheath in curved process, and protrudes beyond its tip.

Third pleopod (fig. 3) in both sexes covering fourth and fifth pair ventrally. Sympod short. Exopod large and operculate with two subequal articles. Distal article obliquely hinged to proximal; distal margin with a few setae. Inner margin of both articles straight, outer ovate. Endopod covered by exopod. Endopod with a short, basal article, and a long, club-shaped, distal article, about two-thirds as long as exopod.

Fourth pleopod (fig. 4) with a short sympod, and a biarticulate endopod and exopod. Basal article of exopod shaped like the head of a hatchet with the sharp edge out. Distal article ensiform, articulated as handle of "hatchet." Basal article of endopod small, outer club-shaped, slightly longer and wider than that of third pleopod.

Fifth pleopod (fig. 5) with a small sympod, a greatly reduced ensiform exopod, and an endopod much the same as in fourth pleopod only slightly smaller and wider.

Type locality.—Fresh-water well near Kelseyville, Lake County, California.

As this goes to press, additional specimens of both sexes and different sizes were received from Mr. Tuttle through Mr. Barnard. These present *only* the following noteworthy variations from the type: Largest specimen (male) 11 mm. long, 2.1 mm. wide. Second antennae as long as the body. Uropods 8 mm. in length with peduncle 5.5 mm., endopod 3.5 mm., and exopod 1 mm. in length. Smallest specimen (2.5 mm.) with second antennae about one-half length of body. Uropods one-fifth length of body, with peduncle and endopod subequal, and exopod three-fourths as long as endopod.

These measurements and other observations indicate that the proportionate lengths of the second antennae and uropods are functions of the age of the individuals, and that in ontogeny, evidently, parts of the uropods develop disproportionately with those parts parallel to the longitudinal axis, the peduncle and endopod, undergoing greater elongation than the exopod which appears relatively short and almost rudimentary in the larger specimens. The significance of the difference in relative growth rate of the uropods, and the relation of their ontogeny to phylogeny are problems for future consideration.

STATUS OF THE GENUS CAECIDOTEA PACKARD

HISTORICAL DIGEST

The validity of *Caecidotea* Packard has been doubted by various authors. It was created in 1871 to receive a blind species, *C. stygia*, from Mammoth Cave, redescribed by Cope (1872) as *C. microcephalus*. The late Dr. S. A. Forbes (1876) redescribed the species as *Asellus stygius*, uniting the genus *Caecidotea* to *Asellus* Geoffroy.

An adequate review of the ensuing controversy was given by Hay (1903) with several remarks of his own. He was the first to point out the polyphyletic nature of the group. If Packard's assertion be true that the two or three species (known at that time) have arisen independently—and he considers this almost a certainty, then the genus, he reasons, is a "heterogeneous assemblage of species grouped together simply because they happen to resemble each other," which is contrary to the usual conception of a genus as a "natural aggregation of species." The resemblance of species he believes to be a matter of convergence, since nearly all cave-dwelling animals have been affected in the same general way, namely, loss of color, degeneration or loss of eyes with an accompanying hypertrophy of tactile organs, and elongation in many groups, especially in the *Crustacea*. Without knowledge of intermediate forms between surface and true subterranean types, and without "positive knowledge that the genus is not a natural one," he suspended judgment and accepted the genus on the "grounds of convenience."

Tattersall (1921), Ueno (1927), and Creaser (1931) question the validity of the genus. Racovitza (1925), who has worked intensively on the European asellids, remarks, "J' ai étudie cette espèce en vue d' une monographie des Asellides cavernicoles qui doit paraître prochainement. La conclusion à laquelle je suit arrivé est que *Caecidothea* est un mauvais genre, que les formes décrites sous ce nom se rattachent à diverses ligneés du genre Asellus et que *C. stygia* (recte: *Asellus stygius*) est un type très peu spécialisé ayant conserve nombre de caracteres paléogénetiques."

COMPARATIVE STUDY OF GENERIC CHARACTERS

In his monograph on the cave fauna of North America, Packard (1888) gave the following diagnosis of the genus:

> *Generic characters.*—Differs from *Asellus* in the much longer body and absence of eyes, and in the longer and narrower head. The second (larger) antennae are almost as long as the body; in *Asellus* only one-half or a little more than one-half as long as the body; the fifth joint nearly twice as long as the fourth; in *Asellus* it is but slightly longer than the fourth. The legs are also slenderer, the terminal joint much narrower, and the claws much

> smaller. The telson (abdomen) is very long and narrow, oblong, two-thirds as broad as long; whereas in *Asellus* it is not so long as broad, and is much rounded. The mouth parts also present some differences; the lobes of the maxilla are narrower; the penultimate joint of the palpus of the maxillipeds are one-half as thick as in *Asellus*, while the middle joint of the mandibular palpus is not so large and thick, and there are other differences, though of less importance.

A careful survey of these generic characters, at least of the more important, is now pertinent.

Loss of eyes is characteristic of subterranean forms. All the described species of *Caecidotea* are blind except *C. kawamurai* which has rudimentary eyes consisting of a few ocelli. Occasional specimens of *C. stygia* have been reported in which a few facets have been found in place of eyes, indicating, as Hay (1903) points out, a reversion to a recently lost character. If we turn to the genus *Asellus,* we find among the species and varieties six which are blind in correlation with an underground existence, all from Europe or Africa (see table 1). Lepri (Dudich 1925) has reported a blind form of *A. aquaticus.* In many of the species of *Asellus*, the eyes are small or subrudimentary, consisting in some specimens of only three or four ocelli. Thus, the presence or absence of eyes alone cannot be used as a generic diagnostic character. Whether other characters separate the two genera remains to be seen.

By dividing the body length by the greatest width, we obtain a "body index" (table 1) for use in comparing the species of the two genera. (Measurements for calculation of indices in table 1 were taken from author's descriptions.) The body indices of the described species of *Caecidotea* range from 4.0 to 7.0, exceeding those of the species of *Asellus* which range from 2.3 to 4.1. A closer study, however, reveals that those forms of *Asellus* which have the larger indices, that is, those which are more elongate, are mostly the subterranean forms, all of which have indices of 4.0 at least. This supports the contention of Hay that elongation is a feature of subterranean existence. Between the extreme indices is an intergrading series of forms.

Using a head index (length over width) we note that the heads of the species of *Caecidotea* are longer in proportion to width than those of the species of *Asellus,* but that two of the blind forms, *A. gauthieri thermonyctophilus* and *A. forelii,* have elongate heads (see table 1). Here again there is a series of intermediate forms between the two extremes.

Comparing the relative lengths of the second antennae with an antenna index (see table 1), derived by dividing the antenna length by the body length, we find that the correlation between subterranean habitat and elongation is not so pronounced as in other characters; for although it is true that the described species of *Caecidotea* have relatively long antennae, several surface species of *Asellus* bear long antennae. The statement of Packard that the antennae in *Asellus* are "only one-half or a little more than one-half as long as the body" does not hold for ten of the species of *Asellus,* and possibly others

TABLE 1

Type	Eyes	Body index	Head index	Second antenna index	Pleotelson index
Caecidotea stygia	Absent	5.0	1.2	0.7	1.5
Caecidotea nickajackensis	Absent	7.0	0.8	0.7	1.5
Caecidotea richardsonae	Absent	4.5	0.6	1.0	1.3
Caecidotea smithsii	Absent		1.1	1.0(?)	
Caecidotea alabamensis	Absent	6.0	0.7	0.6	1.7
Caecidotea kawamurai	Present (reduced)	6.5	0.6	0.5	1.6
Caecidotea tridentata	Absent	5.7	0.7		1.6
Caecidotea akiyoshiensis	Absent	4.0	0.6	0.8	1.1
Caecidotea antricola	Absent	4.4	0.6	2.0	1.3
Asellus aquaticus	Present	2.8	0.5	0.7	0.9
Asellus aquaticus freibergensis	Present (small)	2.7	0.5	1.0	0.7
Asellus aquaticus cavernicolus	Present				
Asellus arthobranchialis	Present			0.8	0.9
Asellus meridianus	Present				
Asellus cavaticus	Absent	4.1	0.4	0.8	1.1
Asellus forelii	Absent	4.0	1.0	0.5	
Asellus coxalis	Present	3.5	0.6	0.7	0.8
Asellus coxalis peyerimhoffi	Present	3.0			
Asellus coxalis africanus	Present				
Asellus coxalis cyanophilus	Present				
Asellus banyulensis	Present	4.1		0.8	
Asellus banyulensis wolfi	Present				1.0
Asellus spelaeus	Absent	4.0	0.5	0.7	1.0
Asellus spelaeus aquae-calidae	Absent				
Asellus hypogaeus	Absent	4.0	0.6	0.8	1.0
Asellus gauthieri	Present	4.0	0.5		1.0
Asellus gauthieri thermonyctophilus	Absent	4.0	0.8		1.3
Asellus polychaetus	Present				1.0
Asellus italicus	Present				1.0
Asellus aquaticus cracoviensis	Present	3.0			
Asellus angarensis	Present	2.8	0.5	0.4	0.9
Asellus baicalensis	Present	2.7	0.6	0.6	1.0
Asellus minutus				1.0	
Asellus dybowskii	Present		0.5	3.0	±1.0
Asellus korotnewskii				1.0	
Asellus goplanus					0.9
Asellus goplanus switzesiana					
Asellus communis	Present	3.5	0.5	0.6	0.9
Asellus intermedius	Present	3.0	0.6	0.7	1.0
Asellus brevicauda	Present	3.0	0.4	0.6	0.8
Asellus hoppinae	Present	2.3	0.5	0.9	0.8
Asellus attenuatus	Present	2.7	0.4	1.0	0.9
Asellus tomalensis	Present	3.3	0.5	0.7	1.0
Asellus californicus	Absent	5.2	0.8	0.8	1.2
Asellus nipponensis	Present	3.3	0.7		1.0

for which I have no measurements, which have antennae three-fourths or more as long as the body. There is considerable overlapping in this feature of the two genera, as a comparison of the indices shows, so that this character can hardly be used in diagnosis. One must also remember that antennae length varies with age and sex. In computing the antenna index, the longest measurement of the antennae obtainable was used.

The last important character listed by Packard that we will consider is the pleotelson. The same general features noted in the characters already discussed are found here also. The pleotelson is proportionately longer in the subterranean types of both genera, and here again there is an intergrading, overlapping series of forms between the extremes (see table 1, pleotelson index).

Thus we see that body proportions as exemplified by proportionate length of body, head, antennae, and pleotelson cannot be used as a reliable diagnostic character, first, because there are among the known forms of the two genera all variations in shape which make a series of connecting forms that intergrade, and secondly, because the elongation characteristic of the species of *Caecidotea* is not exclusive, but is found also in the subterranean forms of *Asellus* and in other underground groups.

Turning now to an examination of certain other structural features, we do not find differences which could be considered generic. Bovallius (1886) indicates the importance of pleopod structure in this connection when he states that "*Caecidotea* seems to be closely connected with *Asellus forelii* and *A. cavaticus,* but, without any knowledge of the form of its pleopods, it is impossible to say whether it ought to be united with *Asellus* or not." Another character, generally regarded as important by systematists, is the number of setae on the inner lamina of the first maxilla. As a matter of fact, the two characters just mentioned are the main ones used by Dudich (1925) in proposing two subgenera for the genus *Asellus.*

The presence or absence of an apophysis on the basal article of the endopod of the second pleopod of the male is a significant character. Of the European species of *Asellus, A. aquaticus, A. a. freibergensis, A. a. cavernicolus,* and *A. arthobranchialis,* have such a process, while all the others, including the blind forms, do not. *A. nipponensis* (Nicholls, 1928) and *Caecidotea kawamurai* from Japan resemble *A. aquaticus* (also reported from Japan) in having this structure and in the general features of the whole appendage. Unfortunately *C. akiyoshiensis* is known only from female specimens. Of the American species of both genera, only our new species bears the structure in question so far as we can determine. (We have no knowledge of this pleopod in *Asellus hoppinae, A. attenuatus, A. tomalensis, Caecidotea richarsonae,* and *C. smithsii.*)

There is also no generic difference in the number of setae on the inner lamina of the first maxilla. Those forms with an apophysis on the second pleopod of the male have four setae, except *Asellus californicus* and *Caecidotea kawamurai,* which are like those without in having five setae.

On the basis of these two characters, as well as many others, we see that species of *Caecidotea* are more closely allied to species of *Asellus* than to species in their own genus. This fact in no way conflicts with, but rather substantiates, the affinities of certain species of *Caecidotea* with species of *Asellus* living in the same regions, which many authors have reported. We are anxious to get specimens of *Asellus tomalensis* from California to compare with our species on this point.

In the observations appended to the description of *A. californicus,* we note that the uropods in the larger specimens are almost three-fourths as long as the body, a condition which approaches that shown in Creaser's (1931) "orphaned species" from Missouri, *Caecidotea antricola,* in which the uropods are as long as the body. In respect to other more fundamental and less variable characters such as those just discussed, however, our Californian species shows more similarity to the Japanese species. This may prove of some significance in an understanding of the origin and affinities of the species.

CONCLUSION

Thus the lack of generic structural differences coupled with intergradations in structural proportions in the two genera forms one basis for declaring *Caecidotea* invalid.

Again, on theoretical grounds, we must agree with Hay that *Caecidotea* does not conform to the generally accepted idea of a genus as a natural aggregation of species, for it is practically assured that the several species have had independent and different origins, and that the resemblances arise from similarity of environment, however brought about.

For the reasons above given, it is no longer feasible to retain *Caecidotea,* and it is time to unite it definitely with *Asellus,* as Forbes advocated in 1876.

SUMMARY

A new blind isopod, *Asellus californicus,* is described from specimens taken from a fresh-water well near Kelseyville, Lake County, California.

After a comparative study of *Caecidotea* Packard and *Asellus* Geoffroy, the former grouping of our subterranean Asellid species is found to be invalid, and the group of species under the name *Caecidotea* is transferred to *Asellus.*

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EXPLANATION OF FIGURES

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Asellus californicus

Figs. 1 and 2. First and second pleopoda of male. × 50.

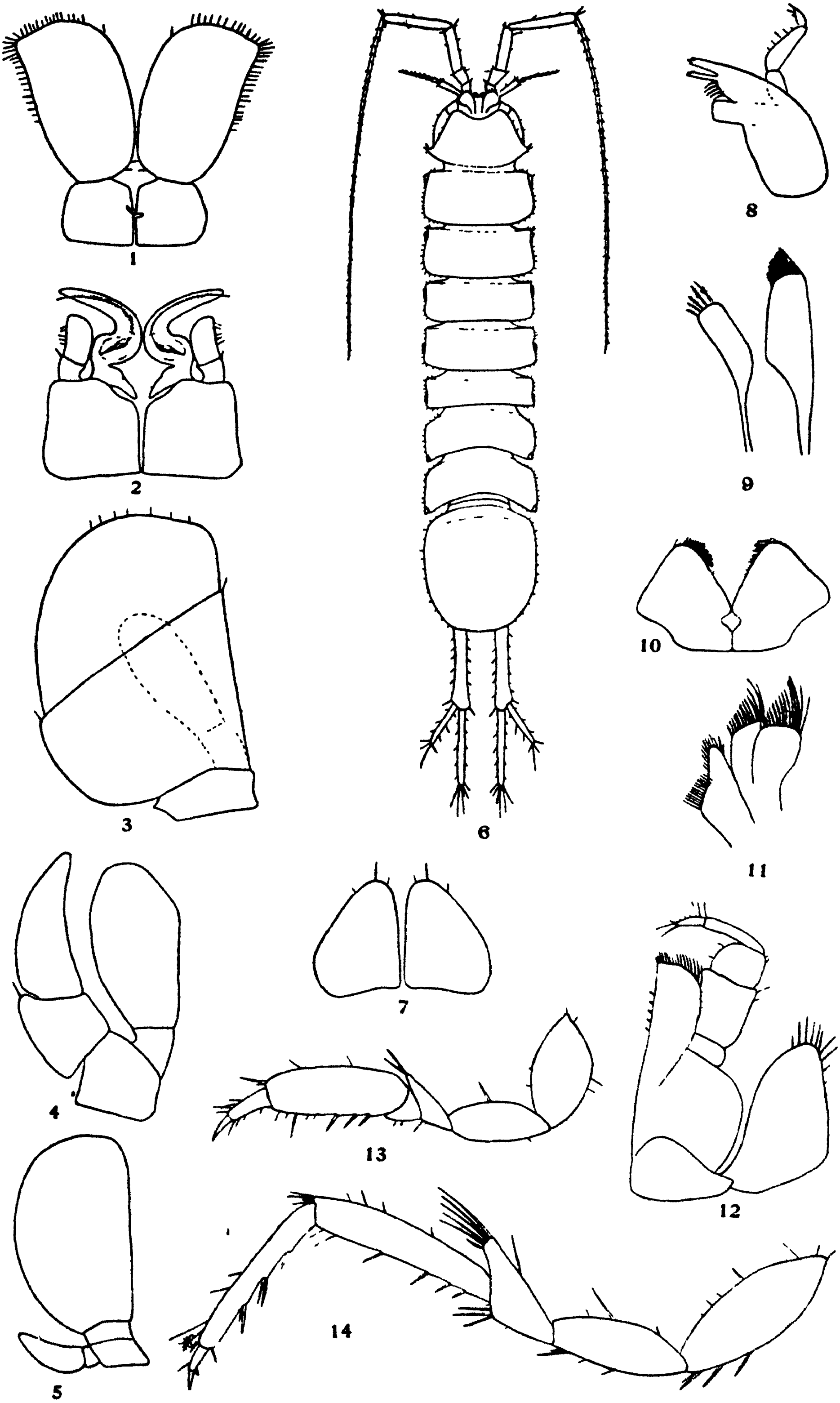
Figs. 3, 4, and 5. Third, fourth, and fifth pleopoda of female. × 50.

Fig. 6. Dorsal view of male. × 12.5. Fig. 7. First pleopoda of female. × 50.

Fig. 8. Mandible. × 50. Fig. 9. First maxilla. × 50.

Fig. 10. Lower lip. × 50. Fig. 11. Second maxilla. × 50.

Fig. 12. Maxilliped. × 50. Figs. 13 and 14. First and seventh leg of male. × 37.5.



Figs. 1–14. *Asellus californicus.* All figures drawn with aid of microphotography

THE ASSOCIATION OF THE TERMITES, KALOTERMES MINOR, RETICULITERMES HESPERUS, AND ZOOTERMOPSIS ANGUSTICOLLIS WITH FUNGI

BY

ESTHER C. HENDEE

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INTRODUCTION

Termites are often reported as occurring in wood which also shows evidence of the work of fungi. Questions of both economic and biological interest center around this association of termites and fungi. Are fungi always present in wood which is infested by termites? Is this association due merely to a similarity of habitat, or do termites and fungi benefit each other? Do termites perhaps aid in the spread of fungi? Does the presence of fungi in the wood make it more attractive to the termites?

With some tropical termites it has been known for some time that a beneficial relationship with fungi does exist, the termites even cultivating certain fungi and then utilizing them for food. The association of North American termites with fungi, however, has thus far received little attention.

Banks and Snyder (1920) reported bands of a fungus growing parasitically on the abdomen and legs of *Reticulitermes flavipes* and *R. virginicus*. This fungus was subsequently described as a new genus and species, *Termitaria snyderi* (Thaxter, 1920).

Dickman (1931) obtained abundant growths of cellulose-digesting organisms in media inoculated with the woody material of termite nests and the fecal pellets of termites. He observed both molds and bacteria growing on the cellulose media, but did not isolate any of them. He obtained no evidence of cellulose digestion in media inoculated with the intestinal contents of either *Reticulitermes* or *Termopsis,* although in some cases there was some mold growth.

The present investigation has involved a survey of the kinds and numbers of fungi associated with fifteen colonies of *Kalotermes minor,* twelve colonies of *Reticulitermes hesperus,* and twelve colonies of *Zootermopsis angusticollis* in their natural habitats. The purposes were: (1) to determine what fungi are most commonly associated with each of these three species of termites; (2) to learn whether any particular fungus or group of fungi is always associated with a given species of termite; (3) to study the distribution of the fungi in and on the termites, in the fecal pellets of *K. minor* and of *Z. angusticollis,* in the frass with which *R. hesperus* stops up abandoned galleries, and in the wood enclosing the burrows of all three species; and (4) to seek some indication of the rôle of termites in the spread of fungi. It is hoped that the results of this work may offer a basis for further investigation of the significance of the association of termites and fungi.

The investigation was undertaken at the suggestion of Professor Charles A. Kofoid. The author expresses her gratitude to Professor S. F. Light and to Professor Kofoid for their interest and many stimulating suggestions during the course of the work. She deeply appreciates the help of Professor Lee Bonar in matters of mycological technique and his careful supervision of the identification of the fungi. She is also indebted to Professor Emanuel Fritz for the identification of the woods in which the termite colonies occurred.

MATERIALS AND METHODS

The species of termites chosen for study represent the three important habit types: *Kalotermes minor* Hagen, the dry wood termites; *Zootermopsis angusticollis* Hagen, the damp wood termites; and *Reticulitermes hesperus* Banks, the subterranean termites.

Tables 1, 2, and 3 give the localities in which the termite colonies were collected, the species of wood in which they occurred, and comments on the state of the wood as regards decay or soundness. With the exception of Alhambra, which is in southern California, all the places mentioned are in the San Francisco Bay region. A question mark following the species of wood indicates that the wood was so badly decayed that it could not be identified with certainty.

Large pieces of wood containing the termite colonies were removed from their sites in the field with as little cutting and handling as possible, and brought to the laboratory in closed containers or wrapped tightly in paper. In cases where a relatively small piece of wood was obtained or where termite burrows were greatly exposed the pieces of wood were placed in sterile metal containers immediately upon removal from their sites in the field. Usually the cultural work was done as soon as the wood containing the termite colonies reached the laboratory. In the few cases where delay was unavoidable the pieces of wood, still in their containers, were left out of doors in the shade until used.

A modification of Leonian's agar (Leonian, 1924) was used as a medium for the fungous cultures. The formula was as follows:

Glucose	6.0 grams
Malt extract (dessicated "Difco")	6.0 grams
Dihydrogen potassium phosphate	1.0 gram
Magnesium sulfate	0.5 gram
Distilled water	1000 cubic centimeters
Agar	20.0 grams

The agar was dissolved in 500 cc of the water and mixed with a solution of the chemicals in the other 500 cc of water. It was then cleared, filtered, and autoclaved at 10 pounds' pressure for 15 minutes. About 25 cc of the medium was poured into Petri dishes, allowed to harden, and then inverted to prevent the accumulation of water of condensation on the surface of the agar. This medium was chosen because it is one in which nearly all fungi grow well.

During the course of the experimental work two hundred fifty-eight cultures were made from the fifteen colonies of *Kalotermes minor,* one hundred twenty from the twelve colonies of *Reticulitermes hesperus,* and one hundred twenty from the twelve colonies of *Zootermopsis angusticollis.* In speaking of making a culture from a termite colony, the term, "colony," is used to designate not only the termites, but also the wood of the walls of their burrows, the fecal pellets of *K. minor* and *Z. angusticollis,* and the frass with which *R. hesperus* stops up abandoned burrows. In connection with the description of each series of cultures, it will be stated from which of these sources the cultures were taken in the case of each termite colony.

The pieces of wood containing the termite colonies were carefully cut open in the laboratory. All instruments used were sterilized by dipping them in 95 per cent alcohol and flaming them over a Bunsen burner. Each culture from wood, frass, pellets, or termites was made immediately upon opening the burrow containing the source from which that particular culture was to be made. In the cases of cultures from wood, frass, or pellets, two inoculations were made upon each plate of medium. In the case of cultures from termites, one

TABLE 1

SOURCES OF COLONIES OF *Kalotermes minor*

Colony number	Locality	Species of wood	Apparent state of wood
1	Alhambra	Port Orford Cedar (*Chamaecyparis lawsoniana*)	Sound
2	Alhambra	Western Red Cedar (*Thuja plicata*)	Sound
3	Niles Canyon	Big-leaf Maple (*Acer macrophyllum*)	Medium decay
4	Niles Canyon	Western Sycamore (*Platanus racemosa*)	Medium decay
5	Niles Canyon	Western Sycamore	Sound
6	Niles Canyon	Western Sycamore	Advanced decay
7	Niles Canyon	Western Sycamore	Sound
8	Niles Canyon	Western Sycamore	Medium decay
9	Niles Canyon	Western Sycamore	Sound
10	Niles Canyon	Western Sycamore	Stained
11	Niles Canyon	Western Sycamore	Sound
12	Niles Canyon	Western Sycamore	Sound
13	Niles Canyon	California Buckeye (*Aesculus californica*)	Sound
14	Niles Canyon	California Buckeye	Stained
15	Niles Canyon	Western Sycamore	Medium decay

TABLE 2

SOURCES OF COLONIES OF *Reticulitermes hesperus*

Colony number	Locality	Species of wood	Apparent state of wood
16	Niles Canyon	Coast Live Oak (*Quercus agrifolia*)	Advanced decay
17	Niles Canyon	Western Sycamore (*Platanus racemosa*)	Stained
18	Niles Canyon	Western Sycamore	Medium decay
19	Berkeley	Monterey Pine (*Pinus radiata*)	Advanced decay
20	Berkeley	Douglas Fir (*Pseudotsuga taxifolia*)	Stained
21	Berkeley	Monterey Pine	Advanced decay
22	Berkeley	Monterey Pine	Advanced decay
23	Berkeley	California Buckeye (*Aesculus californica*)	Advanced decay
24	Brookdale	Madroña (?) (*Arbutus menziesii*)	Medium decay
25	Ben Lomond	Coast Live Oak	Advanced decay
26	Skagg's Hot Springs	California Laurel (?) (*Umbellularia californica*)	Advanced decay
27	Ben Lomond	California Laurel	Medium decay

inoculation was made on each plate. The methods of making cultures from the various sources were as follows:

1. Small pieces of wood from the surface of walls of burrows were cut out and transferred to the agar plates.

2. In making the transplants from wood beneath the surface of walls of burrows, the wood was cut open with sterile instruments and care was exercised to prevent contamination from surrounding wood.

3. Pellets and frass were transferred with sterile forceps from the burrows to the plates.

TABLE 3

SOURCES OF COLONIES OF *Zootermopsis angusticollis*

Colony number	Locality	Species of wood	Apparent state of wood
28	Niles Canyon	Western Sycamore (*Platanus racemosa*)	Advanced decay
29	Niles Canyon	Big-leaf Maple (*Acer macrophyllum*)	Advanced decay
30	Berkeley	Coast Live Oak (*Quercus agrifolia*)	Stained
31	Berkeley	Madroña (*Arbutus menziesii*)	Advanced decay
32	Brookdale	Madroña (?)	Advanced decay
33	Swanton	Madroña	Advanced decay
34	Skagg's Hot Springs	Madroña	Medium decay
35	Berkeley	California Laurel (?) (*Umbellularia californica*)	Advanced decay
36	Berkeley	Monterey Pine (*Pinus radiata*)	Advanced decay
37	Berkeley	California Laurel	Advanced decay
38	Berkeley	Willow (*Salix* sp.)	Advanced decay
39	Skagg's Hot Springs	Douglas Fir (*Pseudotsuga taxifolia*)	Advanced decay

4. Cultures from the exterior of termites were made in four ways: (*a*) the termite was allowed to walk across the agar plate, (*b*) the termite was grasped with the forceps and its body rubbed over the plate, (*c*) the termite was washed in sterile water and the washing mixed with the medium before it had solidified, (*d*) the termite was washed in sterile water and the washing streaked across the medium with a sterile planting wire.

5. Before making cultures from the gut, the termites were immersed in tincture of iodine (U.S.P.), those from colonies 1 and 2 for 45–60 seconds, and those from colony 3 for 2 minutes. They were then rinsed in two changes of physiological salt solution. A termite was placed upon a sterile slide and the gut removed by pulling gently upon the posterior end of the abdomen with fine forceps. The gut and its contents were then streaked across the medium. The fact that the Protozoa in the gut of the termites were still active after the termites had been immersed in the iodine for two minutes was taken as an indication that the iodine did not penetrate to the gut sufficiently to affect

the vitality of any fungous spores which might have been present. Excepting the length of time of immersion in iodine, the above method of sterilization is like that of Beckwith and Rose (1929). They immersed the termites in iodine for 45 seconds. Dickman (1931) criticizes the method of Beckwith and Rose as being inadequate for sterilization. In his experiments Dickman sterilized the exterior of termites by immersion in 1–1000 $HgCl_2$ for 4 minutes. Dickman's paper came to the notice of the author after the cultures from colonies 1 and 2 had been made. The efficacy of sterilization of termites which had walked across cultures of fungi by treatment with tincture of iodine for 1 minute was then compared with that by treatment with $HgCl_2$ for 4 minutes. It was found that neither method was absolutely infallible, but that superior wetting and therefore a higher degree of sterilization was obtained by the use of iodine. The use of iodine was continued in connection with the making of cultures from colony 3, the period of treatment with iodine being extended to 2 minutes as an added precaution. Since the chief purpose in making the cultures in this instance was to learn what fungi were carried by the termites, whether on the exterior or in the gut, the results of all cultures made from termites will be reported. Any further investigation of the significance of the intestinal flora must be preceded by further experimentation with methods of sterilization of the exterior of the termites.

Two precautions were taken to reduce to a minimum the opportunities for contamination of the termites, pellets, frass, and wood of the inner surface of burrows from the air of the laboratory, (1) the time elapsing between the exposing of a termite burrow and the making of an inoculation upon the medium was reduced to a matter of a few seconds, and (2) air currents in the room were prevented so far as possible.

To obtain a check upon the possibility of contamination under these conditions the following experiment was performed. One hundred pieces of wood, having a surface comparable to that of the pieces of wood which were used in making cultures for fungi, were soaked in water and autoclaved at 20 pounds' pressure for 30 minutes. They were then transferred, one at a time, to plates of sterile medium, being exposed to the air of the laboratory approximately the same length of time as termites, pellets, frass, and fragments of wood were exposed in making cultures from them. At the end of two weeks' incubation at room temperature, one out of the one hundred cultural attempts had given rise to a colony of a *Penicillium*. The other ninety-nine showed no fungous growth. Due to probable fluctuations in the spore content of the air of the laboratory, the percentage of contamination might vary somewhat from time to time from the 1 per cent indicated by the above experiment. This possibility of error was taken into consideration in planning the numbers of cultures to be made and in interpreting the results.

The plates upon which the cultures from the termite colonies had been made were incubated at room temperature.

The process of isolating the different fungi was usually begun on the third day. The ends of hyphae, which had grown out away from hyphae of other fungi, were cut off and transferred to other plates of sterile medium. Several transfers were sometimes necessary in order to obtain pure cultures of a given fungus. Where one fungus was overgrown by another, the former was isolated by streaking a dilute suspension of spores from the mixed culture over a plate and selecting from fungous colonies which arose in the streak. Isolation from bacteria was accomplished by incubation at low temperatures and by the use of media acidified with lactic acid. Under these conditions, which prevented or hindered bacterial growth, the fungous hyphae grew out beyond the region of bacterial growth and could be cut off and transferred to other plates. The original fungous cultures were watched for a period of two weeks. At the end of that time the covers were removed from the Petri dishes and the cultures examined under low power of the microscope to determine whether any fungi were present which had not thus far been isolated. When pure cultures of the fungi were obtained transfers were made to sterile test tube slants of the modified Leonian's medium.

The fungi which formed reproductive structures on the culture medium were identified to the genus. Some of the sterile fungi were identified as to class by certain vegetative characters. Others could not be identified at all from the vegetative stages which appeared in the cultures. The generic classification as given by Clements and Shear (1931) was followed.

For demonstration of the presence of fungous mycelium in wood, free hand sections were cut from the wood and stained with aqueous safranin and picroaniline blue according to the method of Cartwright (1929). These preparations showed fungous mycelium blue and the lignified walls of the wood cells red. In the examination of crushed pellets, smears from the gut, and parts of the exterior of the termites for fungous mycelium, picroaniline blue stain was used without the safranin.

FUNGI ASSOCIATED WITH THE DIFFERENT SPECIES OF TERMITES

In order to learn what fungi were most frequently associated with *Kalotermes minor, Reticulitermes hesperus,* and *Zootermopsis angusticollis,* to determine whether any particular fungus or group of fungi tended to be associated with a given species of termite, and to compare the numbers of fungi and the amounts of fungous growth associated with the three different species of termites, ten cultures were made from each of twelve colonies of each of the three species of termites. The sources from which the cultures were taken in the various termite colonies are indicated in table 4.

All but one of these three hundred sixty cultures were positive for fungous growth. Fungi belonging to twenty-nine genera were isolated besides fifteen non-sporulating and therefore undetermined fungous cultures. Lists of the fungi and the number of times they were isolated from each colony of *K. minor, R. hesperus,* and *Z. angusticollis* are given in tables 5, 6, and 7. To facilitate comparison, the totals for each species of termite and for all termites are included in each of the three tables.

Of the twenty-nine genera identified, twelve were isolated from colonies of each of the three species of termites which were studied, eleven from colonies of two of the three species and six from colonies of only one of the three species. Those which were isolated from the colonies of only a single species were all of infrequent occurrence with that species.

TABLE 4

Sources of Fungous Cultures

Species of termite	Colony numbers	Number of cultures made for each termite colony from				Total per colony
		Wood of surface of walls of burrows	Wood 1-2 mm. below surface of walls of burrows	Frass	Fecal pellets	
Kalotermes minor..........	4–15	3	3	..	4	10
Reticulitermes hesperus....	16–27	3	3	4	...	10
Zootermopsis angusticollis......	28–39	3	3	....	4	10

Table 8 shows the genera of fungi which were most frequently isolated from the colonies of *Kalotermes minor, Reticulitermes hesperus,* and *Zootermopsis angusticollis.* The fungi included in this list are those which were isolated from at least five out of twelve colonies of at least one of the three species of termites. No one of these fungi was confined to the colonies of a single species of termite. *Penicillium* and *Trichoderma* were most widespread in their distribution among the colonies of all three species of termites. They were, as a rule, also more abundant than other genera of fungi within individual termite colonies.

The differences in the number of genera of fungi isolated from the colonies of the different species of termites were not great. Seventeen genera of fungi were isolated from the twelve colonies of *Kalotermes minor,* twenty-five genera from the twelve colonies of *Reticulitermes hesperus,* and twenty-two genera from the twelve colonies of *Zootermopsis angusticollis.* The number of genera of fungi isolated from the ten cultures made from each individual termite colony ranged from three to eight per colony for *K. minor,* from four to thirteen for *R. hesperus,* and from two to eleven for *Z. angusticollis;* the

TABLE 5

DISTRIBUTION OF FUNGI AMONG COLONIES OF *Kalotermes minor* WITH TOTALS FOR THE THREE SPECIES OF TERMITES STUDIED

Genus of fungus	Number of times fungus was isolated from termite colonies															
	Colony number												Total for			
	4	5	6	7	8	9	10	11	12	13	14	15	*K m*	*R h*	*Z a*	*all*
Phycomycetes																
Absidia														4	16	20
Mucor			1				1		1				3	18	14	35
Mortierella														12	25	37
Cunninghamella														1		1
Ascomycetes																
Sordaria														10	3	13
Fungi Imperfecti																
Oöspora	8	5	1			3	1	1			3		22	1		23
Monilia														2	4	6
Gliocladium														9	3	12
Penicillium	2	4	6	14	12	7	7	6	10	9	7	8	92	60	82	234
Oedocephalum			1										1	6	16	23
Hyalopus									2				2	1	1	4
Cephalosporium														7	9	16
Haplotrichum				4		1				6			11	4	8	25
Trichoderma		3	5	1	5	2	2	2	1	2	1		24	67	65	156
Botryosporium											1		1			1
Cylindrocephalum	3												3	3	6	12
Spicaria		1											1			1
Verticilliopsis															1	1
Acrostalagmus														8	3	11
Botrytis					2						2		4	3	4	11
Sporotrichum		1								1			2			2
Hormodendrum		2											2	3		5
Hormiactella												1	1	17	1	19
Catenularia							2	2					4	11	17	32
Leptographium														20	9	29
Chloridium														4	5	9
Alternaria														5		5
Fusarium											1		1	2	2	5
Tilachlidium		3			2	5		2	3		4	2	21	18	2	41
Basidiomycetes										2			2	4	1	7
Other cultures (non-sporulating)	6'		3					2			1	4	16	11	4	31

TABLE 6

Distribution of Fungi Among Colonies of *Reticulitermes hesperus* With Totals for the Three Species of Termites Studied

Genus of fungus	Number of times fungus was isolated from termite colonies															
	Colony number												Total for			
	16	17	18	19	20	21	22	23	24	25	26	27	*R.h.*	*K.m.*	*Z.a.*	all
Phycomycetes																
Absidia	....	....	....	1	....	1	1	....	....	....	....	1	4	....	16	20
Mucor	2	....	2	2	....	2	1	3	....	2	4	....	18	3	14	35
Mortierella	....	....	1	....	....	....	3	1	1	6	....	....	12	....	25	37
Cunninghamella	....	....	....	....	....	....	....	....	....	1	....	....	1	....	.....	1
Ascomycetes																
Sordaria	....	....	....	2	....	....	8	....	....	....	....	....	10	....	3	13
Fungi Imperfecti																
Oōspora	....	1	....	....	....	....	....	....	....	....	....	....	1	22	....	23
Monilia	....	....	....	1	....	....	....	....	....	. .	....	1	2	....	4	6
Gliocladium	....	....	....	5	....	3	....	....	....	....	1	....	9	....	3	12
Penicillium	....	7	5	6	1	9	5	5	2	11	6	3	60	92	82	234
Oedocephalum	....	....	....	1	....	1	1	3	. .	....	....	....	6	1	16	23
Hyalopus	....	1	....	....	....	....	....	....	....	....	....	....	1	2	1	4
Cephalosporium	....	....	....	....	....	....	....	....	3	2	....	2	7	....	9	16
Haplotrichum	....	....	1	2	1	....	....	....	....	2	....	....	6	11	8	25
Trichoderma	8	2	....	9	1	7	6	8	7	8	6	5	67	24	65	156
Botryosporium	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1
Cylindrocephalum	....	....	....	....	....	....	....	....	3	....	....	....	3	3	6	12
Spicaria	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1
Verticilliopsis	....	....	....	....	....	....	....	....	....	....	. .	....	....	....	1	1
Acrostalagmus	....	1	....	. ..	....	....	1	....	....	3	2	1	8	....	3	11
Botrytis	....	2	....	....	....	1	....	....	....	....	....	....	3	4	4	11
Sporotrichum	....	....	....	....	....	....	....	....	....	....	....	....	....	2	....	2
Hormodendrum	....	1	....	2	....	....	....	....	....	....	....	....	3	2	....	5
Hormiactella	....	....	....	....	....	6	8	2	....	1	....	....	17	1	1	19
Catenularia	....	....	....	1	....	3	....	5	....	....	2	....	11	4	17	32
Leptographium	....	....	....	2	....	7	5	2	3	....	1	....	20	....	9	29
Chloridium	....	....	....	....	....	....	....	....	1	....	3	....	4	....	5	9
Alternaria	....	....	....	....	....	....	....	1	2	....	1	1	5	....	....	5
Fusarium	....	....	....	....	2	....	....	....	....	....	....	....	2	1	2	5
Tilachlidium	....	....	4	1	....	....	....	....	2	....	6	5	18	21	2	41
Basidiomycetes	1	....	....	....	....	....	....	....	....	....	2	1	4	2	1	7
Other cultures (non-sporulating)	2	....	1	....	7	1	....	....	1	....	....	....	11	16	4	31

TABLE 7

DISTRIBUTION OF FUNGI AMONG COLONIES OF *Zootermopsis angusticollis* WITH TOTALS FOR THE THREE SPECIES OF TERMITES STUDIED

Genus of fungus	Number of times fungus was isolated from termite colonies															
	Colony number												Total for			
	28	29	30	31	32	33	34	35	36	37	38	39	*Z.a.*	*K.m.*	*R.h.*	all
Phycomycetes																
Absidia	....	....	....	2	6	6	....	....	....	....	....	....	16	....	4	20
Mucor	....	1	1	....	....	....	3	....	1	5	3	....	14	3	18	35
Mortierella	....	5	3	4	1	2	....	....	4	2	....	4	25	....	12	37
Cunninghamella	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	1
Ascomycetes																
Sordaria	....	....	....	....	....	....	....	....	....	....	....	....	3	....	10	13
Fungi Imperfecti																
Oōspora	....	....	....	....	....	....	....	....	....	....	....	....	....	22	1	23
Monilia	....	....	....	....	1	2	....	....	1	....	....	....	4	....	2	6
Gliocladium	....	....	....	....	....	....	....	1	1	....	....	1	3	....	12	12
Penicillium	1	7	8	8	10	6	7	10	....	8	7	10	82	92	60	234
Oedocephalum	....	....	....	3	2	1	2	....	....	5	3	....	16	1	6	23
Hyalopus	....	1	....	....	....	....	....	....	....	....	....	....	1	2	1	4
Cephalosporium	....	....	....	....	....	....	....	....	....	9	....	....	9	....	7	16
Haplotrichum	....	....	....	....	....	....	....	6	....	1	1	....	8	11	4	25
Trichoderma	10	3	3	8	1	3	6	8	7	8	7	1	65	24	67	156
Botryosporium	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1
Cylindrocephalum	....	....	....	....	3	....	....	....	....	....	....	3	6	3	3	12
Spicaria	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1
Verticilliopsis	....	....	....	....	1	....	....	....	....	....	....	....	1	....	....	1
Acrostalagmus	....	....	....	2	....	....	....	....	....	....	1	....	3	....	8	11
Botrytis	....	1	2	....	1	....	....	....	....	....	....	....	4	4	3	11
Sporotrichum	....	....	....	....	....	....	....	....	....	....	....	....	....	2	....	2
Hormodendrum	....	....	....	....	....	....	....	....	....	....	....	....	....	2	3	5
Hormiactella	....	....	1	....	....	....	....	....	....	....	....	....	1	1	17	19
Catenularia	....	....	2	9	3	1	....	1	1	....	....	....	17	4	11	32
Leptographium	....	....	....	7	....	....	2	....	....	....	....	....	9	....	20	29
Chloridium	....	1	....	2	....	....	....	....	....	1	....	1	5	....	4	9
Alternaria	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	5
Fusarium	....	....	2	....	....	....	....	....	....	....	....	....	2	1	2	5
Tilachlidium	....	....	1	....	....	....	....	1	....	....	....	....	2	21	18	41
Basidiomycetes	....	....	....	....	....	....	....	....	....	....	1	....	1	2	4	7
Other cultures (non-sporulating)	....	....	....	....	1	....	2	....	....	....	....	1	4	16	11	31

average per colony being 5.08 for *K. minor,* 8.75 for *R. hesperus,* and 7.25 for *Z. angusticollis.*

The differences in amount of fungous growth noted in the colonies of the different species of termites were more distinctive, colonies of *Kalotermes minor* showing less luxuriant fungous growth than those of *Reticulitermes hesperus,* or *Zootermopsis angusticollis.* This was indicated (*a*) by the differences in the abundance of growth resulting from individual inoculations of wood, pellets, or frass onto the nutrient medium; (*b*) by the differences in the total number of isolations from the one hundred twenty cultures made from the twelve colonies of each of the three species of termites, the numbers being two hundred thirteen for *K. minor,* three hundred thirteen for *R. hesperus* and three hundred one for *Z. angusticollis;* and (*c*) from the macroscopic and microscopic examination of the wood enclosing the termite burrows. The wood containing the burrows of *K. minor* sometimes revealed no indication of the fungi upon macroscopic examination. In some cases stains or structural defects, probably due to fungous attack, were visible, but in no case was there sufficient growth for the fungus itself to be visible to the naked eye. It could be revealed only by staining thin sections of wood and subjecting them to microscopic examination. In the colonies of *R. hesperus* and of *Z. angusticollis,* on the other hand, there was often a mat-like fungous growth at certain places on the walls of burrows or a web-forming growth over the fecal pellets or frass. The smaller amount of fungous growth associated with *K. minor* was what would be expected from the relatively greater dryness of the woods containing the colonies of that species as compared to that of those containing the colonies of *R. hesperus* or of *Z. angusticollis.*

TABLE 8

FUNGI MOST FREQUENTLY ISOLATED FROM TERMITE COLONIES

Genus of fungus	Number of termite colonies from which fungus was isolated			
	Kalotermes minor	*Reticulitermes hesperus*	*Zootermopsis angusticollis*	Total
Penicillium	12	11	11	34
Trichoderma	10	10	12	33
Mucor	3	8	6	17
Tilachlidium	7	5	2	14
Mortierella	...	5	8	13
Catenularia	2	4	6	12
Oedocephalum	1	4	6	11
Leptographium	....	6	2	8
Oöspora	7	1	....	8
Acrostalagmus	....	5	2	7

CLASSIFICATION AND NATURE OF THE FUNGI

Fungi belonging to the four classes, Phycomycetes, Ascomycetes, Fungi Imperfecti and Basidiomycetes were found in association with each of the three species of termites (see tables 5, 6, and 7).

Four genera of Phycomycetes were represented. Of these, *Mucor* and *Mortierella* were among the more commonly occuring fungi, as may be seen in table 8. The Phycomycetes are lower filamentous fungi and common saprophytic inhabitants of soil and of decayed wood. Although wood-inhabiting, they are not typically wood-destroying forms. They follow spaces in the wood and seldom, if ever, penetrate lignified walls or materially affect the structural character of the wood. McBeth and Scales (1913) and Paine (1927) found the species of Phycomycetes which they studied incapable of digesting cellulose.

Only one perfect form of the Ascomycetes was isolated. This was a member of the genus *Sordaria*. It was isolated from a comparatively small number of termite colonies. *Sordaria* is reported as commonly growing on dung. In the present case it was isolated from fecal pellets of termites and from wood enclosing termite burrows.

The large majority of the fungi isolated from the termite colonies are classed among the Fungi Imperfecti. Twenty-four of the genera listed in tables 5, 6, and 7 belong to this class. In addition to these, representatives of four other genera of Fungi Imperfecti were isolated later in a more detailed study of three colonies of *Kalotermes minor* (see table 10). These genera were *Phoma, Aspergillus, Cladobotryum,* and *Acremonium.* The Fungi Imperfecti are a group of miscellaneous fungi which reproduce by conidia and which in many cases have been shown to be stages in the life histories of Ascomycetes. They include many wood-inhabiting forms. Some of them have been reported capable of penetrating lignified cell walls (Gerry, 1923), but usually they follow natural or artificial spaces in the wood. While they are not typically wood-destroying fungi, some of them cause stains in wood. Others live in decayed wood as secondary saprophytes. Many of the Fungi Imperfecti are active in cellulose destruction (McBeth and Scales, 1913). Heukelekian and Waksman (1925) found that a *Penicillium* and a *Trichoderma* which they studied, completely decomposed cellulose and gave CO_2 and fungous mycelium as the only end products. Scales (1915) found twenty-one of the twenty-eight species of *Penicillium* which he tested to be capable of fermenting cellulose.

A comparatively small number of Basidiomycetes were isolated. They seldom produce reproductive structures on artificial media and, therefore, cannot be easily identified. Six of the non-sporulating fungi isolated were recognized as being Basidiomycetes from the clamp-connections which their hyphae showed. They were isolated from one of twelve colonies of *Kalotermes minor,* three of twelve colonies of *Reticulitermes hesperus,* and one of twelve colonies of *Zootermopsis angusticollis.* It is probable that some of the other non-sporulating forms may also have belonged to this class. Among the Basidiomycetes are found the true wood-destroying fungi. These fall into two groups: those which attack principally the lignin of the cell walls, and those which, for the most part, destroy the cellulose. The lignin-dissolving fungi usually cause what are known as white rots; the cellulose-dissolving fungi are responsible for most of the brown rots. Still other Basidiomycetes are secondary saprophytes, developing in wood which is already decayed. The isolation of a basidiomycete from a piece of wood does not necessarily mean that the wood has undergone any considerable decay. The stages of incipient decay are often quite prolonged. Hence, wood which is apparently sound may contain living fungi which in time will effect visible decay. Furthermore, wood which is in late stages of decay may no longer contain the fungi which were responsible for the original decay. It is known that often the hyphae of a fungus disappear from wood when decay reaches a certain stage. They apparently undergo auto-digestion upon exhaustion of their food supply. Often, moreover, a basidiomycete isolated from badly decayed wood is not the primary wood-destroyer, but a secondary saprophyte.

STATE OF THE WOOD CONTAINING THE TERMITE COLONIES

In view of the above facts, it becomes evident that the relation of Basidiomycetes to termites may be somewhat different from that indicated by the results of the isolations. It is of interest, therefore, to consider not only the fungous flora of the wood at a given time, but also the apparent condition as regards soundness or decay.

Five out of the total of fifteen colonies of *Kalotermes minor* were in sound, dry wood, showing no evidence of decay. No Basidiomycetes were isolated from these colonies. Four colonies were located in logs or stumps which were decayed on the outside or had decayed regions, but were apparently sound in the region of the termite burrows. Basidiomycetes were isolated from the wood adjacent to the burrows of two of these colonies. Two colonies were in wood which showed stains which may or may not have been caused by fungi. No Basidiomycetes were isolated from these two colonies. Three colonies were in wood showing a moderate amount of decay, but yielding no Basidiomycetes in culture. The wood containing one colony of *K. minor* was in an advanced stage of decay. The termites were found in a large gallery, the walls of which were so punky that they could be picked apart with forceps. No Basidiomycetes were isolated from this colony.

Two of the colonies of *Reticulitermes hesperus* were in apparently sound but discolored wood, four in somewhat decayed wood, and six in wood which was in a late stage of decay. Basidiomycetes were isolated from two of the colonies of the last group.

One colony of *Zootermopsis angusticollis* was in sound but somewhat discolored wood, and one in wood in an early stage of decay. Ten were in badly decayed wood, and Basidiomycetes were isolated from one of these colonies.

In brief, all three species of termites were occasionally found associated with living Basidiomycetes. *Kalotermes minor,* however, was ordinarily found in wood which had undergone little, if any, structural change from fungous attack. *Reticulitermes hesperus* and *Zootermopsis angusticollis* were usually in wood which had been greatly harmed by wood-destroying fungi, but which, at the time of the termite occupancy, might contain only secondary saprophytes.

RELATION OF THE FUNGI TO SPECIES OF WOOD

The termite colonies which were studied occurred in at least eleven different species of wood. Fungi were always present in the wood adjacent to termite burrows. As may be seen from tables 1, 2, and 3, which show the species of wood in which the termite colonies occurred, and tables 5, 6, and 7, which show the fungi isolated from twelve of these colonies, there was a wide distribution of the more frequently isolated fungi among the different species of wood. Penicilliums, for example, were isolated from all eleven species of wood, Trichodermas from ten species, Mucors from nine, and Mortierellas from seven species.

DISTRIBUTION OF FUNGI WITHIN INDIVIDUAL TERMITE COLONIES

The series of cultures made from termite colonies 4–39 yielded data on the occurrence of fungi in wood of the surface of walls of burrows and in wood at a distance of 1–2 mm. from burrows, in fecal pellets, and in the frass. In addition to this, a more detailed study was made of the three colonies of *Kalotermes minor* designated in table 1 as colonies 1, 2, and 3. The results of cultures from various sources in these three colonies have been combined and are summarized in tables 9 and 10.

Fungi were isolated from all the sources from which cultures were made, namely, the exterior of termites, the gut of termites, their fecal pellets, the frass, wood of the surface of walls of burrows, and wood below the surface of walls of burrows.

TABLE 9

SOURCES OF CULTURES FROM TERMITE COLONIES, 1, 2, AND 3
Kalotermes minor

	Exterior of termites	Gut of termites	Fecal pellets	Wood of surface of walls of burrows	Wood 1-10 mm. below surface of walls of burrows
Number of cultures made	17	32	31	31	27
Number of negative cultures	5	21	2	1	11

TABLE 10

FUNGI ISOLATED FROM VARIOUS SOURCES IN TERMITE COLONIES 1, 2, AND 3
Kalotermes minor

	Number of times fungus was isolated from				
Fungus	Exterior of termites	Gut of termites	Fecal pellets	Wood of surface of walls of burrows	Wood 1-10 mm. below surface of walls of burrows
Mucor	6	1	...	12	....
Sordaria	....	....	16	12	8
Phoma	2	....	1	. .	1
Oöspora	1	....	....	....	....
Monilia	2	. ..	....	....	....
Aspergillus	....	1	.. .	....	....
Penicillium	6	4	14	17	2
Hyalopus	. ..	....	....	.. .	2
Cephalosporium	1	....	....	.. .	..
Trichoderma	1	....	....	1	....
Cylindrocephalum	....	....	....	. .	2
Spicaria	3	....	1	2	3
Cladobrotryum	. ..	....	...	1	....
Acrostalagmus	1	....	1	....	. ..
Acremonium	....	....	....	1	....
Hormodendrum	4	....	1	1	5
Alternaria	....	....	....	2	2
Fusarium	....	....	1	....	....
Tilachlidium	....	1	....	1	....
Basidiomycetes	....	1	....	....	1

A comparison of the results of isolations from the one hundred eight cultures made from wood of the surface of walls of burrows with those from the one hundred eight cultures made from wood 1–2 mm. below the surface in colonies 4–39 is given in table 11.

TABLE 11

DISTRIBUTION OF FUNGI IN WOOD ENCLOSING BURROWS OF TERMITE COLONIES 4–39

Fungus	Number of times fungus was isolated from	
	Wood of surface of walls of burrows	Wood 1-2 mm below surface of walls of burrows
Absidia	8	1
Cunninghamella	1	
Mortierella	15	8
Mucor	10	3
Sordaria	5	5
Oöspora	10	5
Monilia	1	4
Gliocladium	3	3
Penicillium	81	45
Oedocephalum	5	4
Cephalosporium	4	3
Haplotrichum	8	4
Trichoderma	59	32
Botryosporium		1
Cylindrocephalum	9	3
Spicaria		1
Verticilliopsis	1	
Acrostalagmus	5	
Botrytis	3	
Sporotrichum		1
Hormodendrum	4	
Hormiactella	5	3
Catenularia	12	15
Leptographium	11	9
Chloridium	2	4
Alternaria	1	1
Fusarium	3	
Tilachlidium	10	20
Basidiomycetes	2	6
Other cultures (non-sporulating)	6	20
Total number of isolations	284	201

The cultures from the wood of the surface of walls of burrows, in general, showed greater amounts of fungous growth and yielded a larger number of different fungi than those from wood 1–2 mm. from burrows. Of the fungi which were identified to genus eighteen genera were isolated more frequently from the surface of walls of burrows, seven more frequently from the wood below the surface, and three an equal number of times from each region. *Penicillium* and *Trichoderma* were isolated about twice as frequently from the surface as from below the surface. The non-sporulating fungi, including the few which were identified as Basidiomycetes, on the other hand, tended to appear more frequently in cultures from below the surface than in those from the surface.

The wood which contained termite colonies 4–39, previously considered, was usually found to be visibly decayed (see tables 1, 2, and 3). It is therefore of interest to consider an example in which the wood containing the termite colony appeared sound. Such an example is offered by colony 1, which occurred in a Port Orford cedar pole. Wood taken at distances of 1–10 mm. from termite burrows in this pole yielded only four positive cultures out of fifteen. Of these fifteen, the six taken at distances greater than 2 mm. were all negative. The wood of the surface of the walls of the burrows, on the other hand, yielded sixteen positive cultures out of seventeen.

In considering these results, it should be borne in mind that the isolation of a particular fungus from a given piece of wood transplanted onto the culture medium does not necessarily mean that the fungus was actually growing in the exact place from which the wood transplant was obtained. It may have been present merely in the form of spores. This would be more likely to occur within the termite burrows than deep in the wood. Spores from a fungus fruiting at a certain place in a termite burrow might easily become scattered throughout the burrow either by the termites or by possible jarring of the wood during its transport from the field to the laboratory. Hence, the isolation of a greater number of fungi from the surface of the walls of burrows than from wood below the surface of the walls might indicate either a greater mycelial growth on the surface, more abundant fruiting of fungi within the space afforded by the burrows, or even the presence of spores introduced from the exterior by the termites. From the nature of the Phycomycetes and Fungi Imperfecti, which were found in such abundance in the wood lining the termite burrows, and which are known to grow more rapidly through spaces in the wood and to fruit on surfaces of wood rather than deep in wood, it would seem probable that both a greater mycelial growth and more abundant fruiting occurred on the surface of walls of termite burrows than below the surface. In many cases, it was possible to demonstrate this by macroscopic or miscroscopic examination. Some of the termite colonies were in wood which was very damp. The walls of the burrows of these colonies were often covered with a growth of fungi which was easily visible to the naked eye and on which reproductive structures could be detected by means of a hand lens. In the drier wood, especially that in which the colonies of *Kalotermes minor* were located, there was usually not enough fungous mycelium to be apparent macroscopically. Thin sections were therefore cut, stained with safranin and picroaniline blue and subjected to microscopic examination. Figure 1 is a photomicrograph of a section cut from the rough surface of a termite burrow in the pole which contained colony 1. The minute fungous hyphae appear as slender dark lines Other sections were cut at right angles to the surface of walls of burrows in the same pole. They revealed fungous hyphae following the lumina of the cells, although usually not in as great abundance as in sections parallel to and at the surface of walls of burrows. Sections cut from the wood at the ends of burrows in this dry, apparently sound pole, in general, showed decidedly less fungous mycelium than the wood at the sides of older burrows. Sections cut from wood in several regions at distances greater than a centimeter from the termite burrows showed no fungous mycelium. The results, then, of the cultural study and of macroscopic and microscopic examination indicate that fungi are more abundant in wood at the surface of the walls of termite burrows than in wood away from burrows.

Fig 1 Photomicrograph showing minute fungous hyphae in wood of the wall of a burrow of *Kalotermes minor* Section from rough surface of burrow located in the apparently sound, dry wood of a Port Orford cedar pole x 360.

About the same number of different fungi were isolated from the fecal pellets of *Kalotermes minor* and of *Zootermopsis angusticollis* as from the walls of the burrows. Due to the porous character of the pellets, it did not seem practicable to try to sterilize the exterior of pellets before culturing from them. It is therefore to be expected that spores from fungi growing upon the wood enclosing the burrows may have been present on the pellets.

Pellets were included, nevertheless, among the sources from which cultures were taken, because it was desired to have as nearly as possible a complete picture of the fungous flora associated with the termites, and it was thought that there might be fungi present in the pellets which were not present in the wood. In the burrows of *Zootermopsis angusticollis,* which were comparatively damp, there was often a visible web-like growth of fungous hyphae over the pellets. When pellets of either *Z. angusticollis* or *K. minor* were placed upon nutrient agar, hyphae could be observed growing out from the surface of the pellets. Their presence within the pellets was also demonstrated by crushing the pellets and staining them with picroaniline blue.

The frass found in the burrows of *Reticulitermes hesperus* yielded about the same number and kinds of fungi as the wood enclosing the burrows.

Cultures made from the exterior surfaces of seventeen termites of the species *Kalotermes minor* gave positive results in twelve cases (see tables 9 and 10). Termites were immersed in picroaniline blue, rinsed in sterile water, and their body surfaces and appendages then examined microscopically. Fragments of fungous hyphae and conidiophores bearing conidia were observed clinging to the spines on the bodies, legs and antennae, and, in the case of alates, also to the spines on the wings. These fungi did not appear to be growing on the termites, but merely to have become entangled on the spines as the termites walked through patches of mycelium.

Out of thirty-two cultures made from the gut of *Kalotermes minor* eleven positive cultures were obtained (see tables 9 and 10). The fungi isolated from the gut were, with one exception, forms which were also isolated from the wood enclosing the termite burrows. A few smears made from the wall and contents of the gut were examined microscopically, but no fungi were observed in the case of termites taken from their natural habitats. Due to the large bulk of the intestinal contents, a few small spores might easily have escaped observation. Hyphae or germinating spores, if present in any considerable amount, would have been much more likely to be seen. When termites had been fed experimentally upon pure cultures of an *Alternaria,* a fungus having large, dark spores, it was possible to distinguish many entire spores, fragments of spores, and a few fragments of hyphae in the gut. Cultures made from the gut of these termites gave abundant growth of the fungi upon which the termites had been feeding.

RÔLE OF TERMITES IN THE SPREADING OF FUNGI

A comparison was made between the fungous flora of a pole uninfested by termites and that of one infested by *Kalotermes minor.* The poles were both of Western Red Cedar and had similar histories of service. Twelve cultures made from heartwood of the pole uninfested by termites were all negative for fungous growth. Seventeen out of the twenty-seven made from sapwood of the same pole were also negative. From the ten positive cultures, which came from sapwood 1.5–6.0 mm. from the exterior of the pole, or from cracks, two fungi, *Sordaria* sp. and *Alternaria* sp. were isolated. In the termite-infested pole, fourteen cultures taken at intervals throughout the diameter of the pole, seven from wood of the surface of walls of termite burrows and seven from wood 1–2 mm. below the surface of the walls, were all positive. The same two fungi, *Sordaria* sp., and *Alternaria* sp., which were found near the exterior of the pole uninfested by termites were common in the second pole in both sapwood and heartwood adjacent to termite burrows. Nine other genera of fungi were also present, of which the most abundant was *Penicillium.*

In a previous section we noted that fungi were more abundant within termite burrows than in wood away from burrows. Attention was also directed to the opportunity which the spaces of termite burrows offer for more abundant fruiting of Phycomycetes and Fungi Imperfecti and for growth of the mycelia of fungi which do not easily penetrate lignified walls.

The ability of termites to carry fungi was demonstrated by the isolation of fungi from the exterior and from the gut of termites, and by the observation of hyphae, conidiophores, and conidia clinging to the bodies and appendages of termites.

It seems evident, then, that termites may introduce fungi which were not previously present in the wood which they enter, and that they may aid in the spread through the wood of these fungi and of those already present.

GENERAL OBSERVATIONS

The results of this investigation indicate that fungi are associated with *Kalotermes minor, Reticulitermes hesperus,* and *Zootermopsis angusticollis.* They were isolated from every termite colony which was studied. Their presence on the walls of the burrows and in the fecal pellets of the termites was demonstrated. Termites enlarge their burrows; they eat their fecal pellets. Fungi would seem, therefore, to be a common element in the diet of termites. Further investigation, however, will be necessary to indicate the significance of fungi as food for termites.

Certain fungi are capable of causing chemical changes in wood. Hence, the question whether their presence may render the wood more available or more attractive to termites also becomes a problem worthy of study.

SUMMARY

1. Fungi were isolated from each of the fifteen colonies of *Kalotermes minor*, twelve colonies of *Reticulitermes hesperus*, and twelve colonies of *Zootermopsis angusticollis*, which were studied.

2. Representatives of thirty-three genera of fungi and twenty non-sporulating cultures of fungi were isolated from the colonies of the three species of termites.

3. A somewhat smaller number of fungi was associated with *Kalotermes minor* than with *Reticulitermes hesperus* or *Zootermopsis angusticollis*, seventeen genera of fungi being isolated from twelve colonies of *K. minor* as compared to twenty-five genera from twelve colonies of *R. hesperus*, and twenty-two genera from twelve colonies of *Z. angusticollis*.

4. The average number of genera of fungi isolated from ten cultures made from each of twelve colonies of each of the species of termites was 5.08 for *Kalotermes minor*, 8.75 for *Reticulitermes hesperus*, and 7.25 for *Zootermopsis angusticollis*.

5. A smaller amount of fungous growth was noted in the colonies of *Kalotermes minor* than in those of *Reticulitermes hesperus* or *Zootermopsis angusticollis*.

6. There was no evidence of any specific relation between a given species of termite and any genus of fungus.

7. *Penicillium* and *Trichoderma* were the genera of fungi most frequently isolated from the colonies of all three species of termites.

8. The wood containing colonies of *Kalotermes minor* usually showed little structural injury from fungous attack, while that of *Reticulitermes hesperus* and of *Zootermopsis angusticollis* usually showed decay.

9. The fungi most commonly associated with the termites showed no apparent correlation with the species of wood in which the termite colonies occurred.

10. Termites were found to be capable of transporting fungous spores and hyphae.

11. Twelve cultures made from heartwood of a pole uninfested by termites were all negative. Seventeen out of twenty-seven made from sapwood of the same pole were also negative. In a termite-infested pole fourteen cultures taken at intervals throughout the diameter of the pole adjacent to termite burrows were all positive for fungous growth.

12. Fungi were more abundant on the inner surface of termite burrows than in the wood away from burrows, only four cultures out of fifteen taken from wood 1–10 mm. below the surface of burrows of a colony of *Kalotermes minor* being positive as compared to sixteen positive cultures out of seventeen taken from the surface of the walls of burrows.

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STUDIES ON THE STRUCTURE AND LIFE-HISTORY OF OSTIOLUM OXYORCHIS (INGLES) FROM THE CALIFORNIA RED-LEGGED FROG RANA AURORA DRAYTONI

BY

LLOYD G. INGLES

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STUDIES ON THE STRUCTURE AND LIFE-HISTORY OF OSTIOLUM OXYORCHIS (INGLES) FROM THE CALIFORNIA RED-LEGGED FROG, RANA AURORA DRAYTONI

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INTRODUCTION

The importance and significance of life-histories in the class Trematoda can hardly be over-estimated. These parasites infect most of the orders of the vertebrates. They also parasitize the gastropods to such an extent that, as some investigators suggest, even the evolution and spiraling of the characteristic gastropod shell may be attributed to their presence in the livers of these molluscs since the early Cambrian (Sinitsin, 1931). Other groups such as arthropods, annelids, and vertebrates serve these parasites for certain of their stages.

Each family or subfamily seems to have its own type of life-history. The genera of some families exhibit much more specificity for their hosts than do those of others. This host specificity is assuming greater importance as more and more life-histories are investigated. Johnston (1915) has pointed out that it might be used in the study of the distribution of the ancestors of the present hosts. Unfortunately, the investigation of specificity for the hosts calls not only for a proper identification of the hosts involved, but also for an accurate knowledge of their genetic relationships, their life-histories, and finally their ecology, if the distribution of the parasite is to be considered. All these branches of investigation have been neglected on the Pacific Coast in respect of the hosts involved in this study, except *Rana* (see Storer, 1925). Other problems of economic and biological interest which might be solved by studying life-histories in general and specificity in particular are those arising with the introduction of adventitious animal hosts.

It might be added that California offers excellent opportunity for a study of this kind, in that the fresh-water Mollusca are limited to comparatively few species and many introduced vertebrate forms have now become established. The most promising of these studies would concern the investigation of the flukes of the bullfrog, muskrat, and opossum, which have been introduced here from the eastern United States. The evolution of the trematodes, and that of their hosts as well, will become better known as fuller knowledge of their specificity is attained.

The present paper deals with the life-history of ***Ostiolum oxyorchis*** (Ingles) from the California red-legged frog, ***Rana aurora draytoni***. Although Swammerdamm described a fluke from the lungs of the European frog as early as 1737, practically nothing was known of its life-history until the past decade.

Fortunately, the life-histories of two species of frog lung flukes, which Travassos and Darriba (1930) believe to represent different genera, have already been worked out by Krull (1931) in Michigan. It is interesting to compare these life-histories and their larval stages with those of a Californian species which must be included in one of these genera.

Sinitsin (1931) believes the parthenitae are more conservative than are the maritae so far as host specificity is concerned. He explains this by assuming an earlier beginning of parasitic life for the parthenitae. A test of this assumption would call for a thorough knowledge of the life-histories of many types, particularly those closely related forms which are greatly separated in their distribution. If there is a general conservative specificity of the parthenitae for their hosts, then we would expect to find closely related flukes with parthenitae living in closely related molluscs.

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ACCOUNT OF *OSTIOLUM OXYORCHIS* (INGLES)

HISTORICAL

The first record of a fluke from the frog is the description of a lung fluke by Swammerdamm (1752). This parasite, now known as *Haplometra cylindracea* (Zeder), is thus one of the oldest known flukes. Looss (1894) was the first to give descriptions of these flukes from frogs which were of specific value. Looss (1899) gave the name *Haematoloechus* to the group of flukes which parasitize the lungs of anurans. The name *Haematoloecha* had previously been given to an hemipteran by Stäl and because of the similarity in the two names Looss was influenced by Braun to change the name *Haematoloechus* to *Pneumonoeces* (Looss, 1902).

Leidy (1851, 1856) gave a few descriptions of flukes from frogs which are inadequate for specific determination. Stafford (1902) described five additional new species of lung flukes from Canadian frogs in the genus *Haematoloechus,* but later (1905) published them under the following names: *Pneumonoeces longiplexus, P. breviplexus, P. varioplexus, P. similiplexus,* and *P. medioplexus*. Ward (1917) established the genus *Pneumobites* which included the first two of the above mentioned species. Since Stafford had only a few specimens of *H. varioplexus* and since it differed only in egg size, which is variable, from *H. similiplexus,* Cort (1915) was inclined to consider *H. varioplexus* as a *species inquirenda*. Stafford (1905) considered Pratt's *Ostiolum formosum* (1903) to be a synonym of his *medioplexus*. Seeley (1906) described another lung fluke, *Ostiolum complexus* from *Rana pipiens* of North Carolina. Cort (1915) described another species, *O. coloradensis* from the same host species from eastern Colorado. Irwin (1929) described *H. parviplexus* from *Rana clamitans* from Minnesota. The writer (1932) described *H. tumidus, H. kernensis, O. oxyorchis,* and *O. confusus* from the California red-legged frog, *Rana aurora draytoni*. In this paper it will be shown that *O. confusus* is a synonym of *O. oxyorchis*. Other species not found in the United States which have been described are: *H. variegatus* (Rudolphi, 1819), the type species, *H. similis* Looss, 1899, and *H. asper* Looss, 1899, from European frogs; *H. campyristis* (Klein, 1905) from *Rana hexadactyla* of India; *H. schulzei* (Wundsch, 1911), from European frogs; *H. australis* (Johnston, 1912) from *Hyla aurea* and *Limnodynastes peroni* of Australia; *H. neivai* (*Travassos and Artigas,* 1927) from *Leptodactylus ocellatus* of Brazil; finally a new species, *H. fülleborni,* from the lungs of the South American toad, *Bufo marinus,* by Travassos and Darriba (1930). These last authors have revised the group and have revived Pratt's *Ostiolum* to include those flukes living in the lungs of anurans which have the coils of the uterus entirely between the intestinal caeca. They included under the name *Pneumonoeces* all those frog lung flukes in which the intestinal caeca have folds of the uterus lateral to them, thus eliminating Ward's *Pneumobites* (1917). These workers also revived the species name *H. varioplexus* and reduced to a synonym of that name, *H. similiplexus*, because of the priority of the former. Recently (1932) the writer directed attention to a breach of the zoological rules of nomenclature by Looss (1902) in changing the name *Haematoloechus* to *Pneumonoeces*, and revived the name *Haematoloechus* for the name of the genus which Travassos and Darriba (1930) defined as *Pneumonoeces*. Unfortunately, the revision of the genus by these last authors did not come to the writer's attention until his paper describing four new species of *Haematoloechus* had gone to press. One of these species belongs to the revived genus *Ostiolum* and will be treated as such in this paper.

At present thirteen species of *Haematoloechus* and four species of *Ostiolum* are recognized, as follows: *H. variegatus* (*Rudolphi*, 1819) type; *H. similis* Looss, 1899; *H. asper* Looss, 1899; *H. longiplexus* Stafford, 1902; *H. breviplexus* Stafford, 1902; *H. varioplexus* Stafford, 1902; *H. schulzei* (Wundsch, 1911; *H. campyristis* (Klein, 1905); *H. neivai* (Travassos and Artigas, 1927); *H. parviplexus* (Irwin, 1929); *H. fülleborni* (Travassos and Darriba, 1930); *H. tumidus* Ingles, 1932; *H. kernensis* Ingles, 1932; *Ostiolum medioplexus* (Stafford, 1902) type; *O. complexus* (Seeley, 1906); *O. coloradensis* (Cort, 1917); and *O. oxyorchis* (Ingles, 1932).

Von Linstow (1890) described the life history of a genus, *Haplometra*, which is closely related to *Ostiolum* and *Haematoloechus*, but his work was based on morphological similarities of the stages to the adult. Later (1922) Eckstein reported that he had found the metacercaria of *H. variegatus*. Van Thiel (1930) described the development of *Agamodistomum anophelis* from European *Anopheles maculipennis* from the metacercaria to the adult form, *H. variegatus* (Rud.) in the European frog *Rana esculenta*. This work involved some experimental feeding and gives an excellent account of the development of the parasite from the young forms to the adult. The best and most complete account of the life history of the lung flukes is that by Krull (1931), who proved experimentally and described all the stages of *Ostiolum medioplexus* and *Haematoloechus parviplexus* from the frogs of Michigan. The present paper gives an account of the life history of *Ostiolum oxyorchis* based on laboratory experiments and observations in the natural environments of the frog, snail, and insect hosts from California.

General Methods

The general methods employed in determining the life-history of this species are discussed in this section, while the more detailed methods are to be found in the several parts dealing with the successive stages as considered.

A general survey was made of the country surrounding Berkeley for stations where infected frog hosts could be obtained. From these various ponds and streams the trematode fauna of many frogs was collected and examined with a view to selecting for this study a trematode represented in large numbers. The new species were identified and described (Ingles, 1932). The literature was studied in order to gain a general idea of the life-histories in closely related species, and the start was made by examining a large variety of water-dwelling insects for adolescariae. The life-history stages were studied in the living dragonflies, some dragonflies were mounted for future study, and others were fed to young frogs, the feces of which had been negative for eggs for a period ranging from thirty to fifty days. When a frog was selected for feeding, the adolescariae were dissected out of the insect and forced into the esophagus of the frog with a pipette. Such frogs were fed varying numbers of these adolescariae every few days and the feces were examined microscopically for eggs until they appeared. After eggs appeared in the feces the frog was killed and a careful search was made for flukes. They were always found only in the lungs in such frogs, and ranged from adults down to metacercarial sizes. These flukes were then induced to lay eggs for further study by being placed in distilled water. After most of the eggs were evacuated the flukes were relaxed in cold water or flattened between two slides held apart by coverglasses at the ends and bound by a rubber band. The flukes were then fixed by plunging them into hot Bouin's fluid, where they were left for about three hours. They were stained and mounted in various ways. The best results in staining were obtained by using alum carmine, alum cochineal, and Mayer's hemalum.

It was found that the eggs did not hatch in the laboratory even after several months, and it was assumed that they must require ingestion by a proper parthenita host to effect the hatching. In order to test this hypothesis mollusks were intensively collected from a small but permanent pond. Certain dragonfly nymphs in this pond harbored the metacercariae of the fluke, as had already been shown by experiments. It was therefore suspected that some mollusk in the pond was the parthenita host. Many collecting trips to the pond were made, and four species of Mollusca were taken—a small bivalve, ***Musculium lenticulum*** Gould, and three pulmonates, ***Planorbis*** (***Gyraulus***) ***parvus*** Say, ***Planorbis*** (***Gyraulus***) ***vermicularis*** Gould, and ***Physa gyrina*** Say. These were examined through the fall and winter of 1931–1932 without finding any infection until spring, when one was found that later proved to be that of *Ostiolum oxyorchis.* The infection was found only in *Planorbis parvus* in nature. The eggs of the fluke were fed to the three species of pulmonates for varying periods of time, and the snails were transferred to a clean dish of pond water and watched for the appearance of feces. When the feces appeared they were transferred to a slide and gently rolled beneath a coverglass in normal saline solution. The miracidia were secured in this manner. All the species of snails caused the eggs to open. In order to secure the parthenitae and the cercariae nine snail-feeding experiments were started at various times in May. The snails were fed great numbers of eggs of *O. oxyorchis,* which were placed in a small watch glass or sprinkled on their food. Controls were kept on these experiments. After the snails had been fed they were placed in a jar containing clean pond water with some laboratory-raised algae and dead leaves that had been soaking in the laboratory for some time. This jar and the controls were then covered and placed in about six inches of water in the department's fresh-water pool, so that a more constant temperature was maintained. These snails were examined from time to time for infection by crushing them in a watch glass and examining the contents under the microscope. In this manner the parthenita hosts were discovered and the sporocysts and cercariae were secured earlier than they were found in the naturally infected snails of the ponds.

After all the life-history stages were thus demonstrated the experiment was repeated on hosts, all of which had been raised in the laboratory from eggs, with exception of the frogs, which had been transformed from young tadpoles. Frog tadpoles could not be infected with lung flukes by Krull (1931) nor could they be infected with this fluke by me. The dragonfly eggs were secured and handled as described by Krull (1929). Snail eggs were obtained from sticks and other material in the pond or in the aquaria where the animals were kept.

LIFE-HISTORY STAGES AND EXPERIMENTS

THE EGG AND MIRACIDIUM

The eggs of *O. oxyorchis* average 27μ in length by 17μ in diameter (pl. 11, fig. 5). They are chocolate brown in color and so opaque that the details of the miracidia cannot be seen through their thick shells. They did not hatch in pond water under laboratory conditions during a period extending from July, 1931, to February, 1932. Following this period the miracidia in these eggs do not appear to be shrunken or dead, yet it cannot be asserted positively that they were alive. No movement could be seen in the eggs just after they were laid, but the ciliary motion could easily be seen in those that had passed through the digestive tract of the snails. If the flukes were placed in cold distilled water just after they were taken from the frog, they actually poured out eggs. These eggs were transferred to small stender dishes of pond water, where they would settle to the bottom and adhere to the glass after a few hours. Various artificial methods were tried to induce the eggs to hatch. Some eggs were placed in various dilute solutions of HCl, and others were placed in the opened digestive tract of various snail hosts, but none of these attempts were attended with any success.

The eggs will hatch when they are eaten by any of the three pulmonates which were found in the pond where the infected frogs occurred. The miracidia were obtained by the method already described. The eggs in all three species of snails which opened numbered about 68 per cent of those that were eaten. Miracidia were often seen to emerge while the eggs were being watched under the microscope. The process required about three and one half minutes (pl. 11, fig. 6).

The living miracidia were very slow and sluggish. As a rule they did not swim out of the high power field of the microscope. There were a few, however, that could make some progress while swimming. They were pear-shaped during the process, with the anterior end drawn out into a distinct nipple. The animal spiraled very slowly and was continually extending and contracting. After a few moments the animal would attach itself by the everted nipple-like gut and continue to contract and extend itself even after the cilia had stopped moving. Once when an animal turned end-over-end it was thought that cuticle plates could be seen, but the number or even their presence could not be observed again. In one specimen a distinct notch a short distance from the posterior end indicated the presence of such a plate. The cuticle *is* 1.1μ thick. It was the posterior third of the body that extended and contracted. It seems probable that cuticular plates, if present, may in some way effect this extension. The miracidia were able to cover some distance by crawling.

The miracidia were short-lived. Many were timed as they left the egg, and the longest time that any lived was sixteen minutes. Just before death a large blob of protoplasm appeared at the posterior end. In life the miracidia were opaque, and although a thorough search was made for flame cells they were never observed. By staining with a number of intra-vital dyes a number of features could be observed that could not be seen before. The vital stains found to be most satisfactory were both aqueous and alcoholic solutions of methylene blue, brilliant cresyl blue, and neutral red. When the miracidia were stained intra-vitally there could be seen eight nuclei of varying sizes which showed different degrees of affinity for the dye. In order to study these nuclei more closely, permanent balsam preparations were made of the miracidia by fixing them in Schaudinn's fluid and staining them in iron haematoxylin. During life, eight miracidia averaged 29μ in length and 15μ in width. While swimming, the animals were longer and narrower and one individual in this condition measured 31μ by 12μ. The miracidia shrank considerably after fixation in Schaudinn's fluid. Fifteen animals had an average length of 22μ and a width of 12μ after this process. After this procedure they also lost their typical pear-shaped outline and became egg-shaped (pl. 11, fig. 1).

Three distinct kinds of nuclei can be distinguished among the eight nuclei which appear in all the animals studied. The two that are usually most anterior are round or oval and measure 2.7μ in diameter in the specimen figured (pl. 11, fig. 1). These nuclei stain only fairly heavily and as a rule have only very small patches of dispersed chromatin. They also lack the heavy chromatin periphery that is characteristic of those in the posterior part of the animal.

The second type of nuclei is also represented by a pair, whose members usually are not situated in any definite place in the animal. They are more often median in position, but occasionally a specimen was found with one of the pair anterior to all the other nuclei and sometimes one was posterior to the others. They are the smallest of the eight nuclei and measure about 2.3μ in the specimen figured. They can easily be distinguished from the rest, even with the intra-vitam stains, by the presence of a very large, heavily staining karyosome.

The third type of nuclei is represented by four nuclei, and is characterized by a heavy peripheral border and a somewhat irregular, massive karyosome In most of these specimens the nuclei are unequal in size, ranging from that of the anterior group to a diameter of about 5μ, with the largest one placed posteriorly. In many specimens this large nucleus has a large crescent-shaped karyosome, but in others it is of various shapes. Surrounding it is often seen a heavier staining material.

The function of these various nuclei is not known but it is supposed that the posterior four are concerned with reproduction while the rest may be associated with the gut and excretory system. It is to be noted that the nuclei differ in number and arrangement from those described by Krull (1931) for the miracidia of *O. medioplexus*.

In addition to the nuclei very little can be seen except a few scattered granules and fibers which radiate back from the anterior end. These fibers give the eggs a characteristic appearance while they contain the miracidia.

The animal is covered with cilia which spiral in a clock-wise direction when viewed anteriorly. These cilia begin near the everted part of the gut and are here very short. They become progressively longer as they approach the posterior end. About the middle of the body they are about 6–7μ in length. At the posterior end no actual cilia can be seen, but little stubs are present which may represent them.

Two methods were decided upon to determine whether or not the snail's gut is actually penetrated by the miracidium. Since the miracidia are so short-lived and swim so poorly it hardly seems credible that they could penetrate the snail from the outside. It is of course possible that they could be drawn by the snail into its respiratory aperture and penetration effected there. After the snails belonging to all three species had been fed on the eggs of *O. oxyorchis* for several hours they were dissected and the guts placed on a slide under a coverslip in normal saline solution. These guts were so transparent that the eggs and miracidia could be seen through the gut wall. The miracidia were seen to attach themselves to the wall but were not active in penetrating it. In a small *Planorbis parvus* one miracidium was seen with its anterior end well imbedded in the wall of the gut. It was impossible to see whether the cilia of the miracidium were active or not because of the intense ciliary action in the intestine.

The second method was to dissect the guts from the snails and fix, stain, and section them serially for examination. Sections were made of the guts of two young *P. parvus,* one of which had been fed the eggs of *O. oxyorchis* six hours before and the other three days before. These sections were stained with Delafield's haematoxylin and carefully examined under the oil immersion. In the six-hour gut several miracidia could be seen attached to the wall, but no evidence of penetration was found. In the snail that had been fed three days before there was one unquestioned occurrence of a miracidium completely buried in the wall of the gut (pl. 11, fig. 4). The section was cut in such a way that all eight nuclei could be distinguished, and even the different types were evident. There were a few other instances of penetration in the gut wall, but none that could not be questioned. Thus the evidence that has been secured indicates that the miracidia normally penetrate the wall of the gut perhaps by means of some kind of cytolytic secretion.

THE SPOROCYST

From 50 to 60 days after *Planorbis parvus* and *P. vermicularis* had been fed the eggs of *Ostiolum oxyorchis* they began to discharge mature cercariae. After the snails had passed the fortieth day bodies representing doubtless the parthenita generation were discernible in the liver. If the snails are opened on about the fiftieth day these sporocysts will be found to be of various sizes and to contain cercariae in various degrees of development (pl. 12, figs. 9, 15, 16). The sporocysts are usually oval or egg-shaped and when young may contain as many as ten germ balls, each of which is presumably a developing cercaria. As they increase in size these immature cercariae decrease in number until they become active, when there are usually two, or seldom three, left in the sporocyst. Even when the cercariae are mature, the sporocysts are seldom more than 0.250 mm. long by 0.125 mm. wide. Their walls are very elastic and may be stretched to great lengths by the mature cercariae without breaking. They were found only in the digestive gland of *P. parvus* and *P. vermicularis.*

In the experiments about 65 per cent of all the *P. parvus*, but less than 5 per cent of the *P. vermicularis* were infected. This difference might be attributed entirely to different degrees of specificity, but might also be accounted for if snails of different ages were used for each of the species. It was noticed that the largest individuals of *P. parvus* were seldom infected, and since no *P. vermicularis* were raised for the experiment it is possible that the snails of this species which were used in the experiments were all older than the *P. parvus* and therefore more difficult to infect. During the spring of 1932 over 400 *Physa gyrina* were examined from the ponds about Berkeley and none was infected with xiphidiocercariae. None of over 150 *P. vermicularis* was infected. Only 4 per cent of the *P. parvus* showed infection when these species were collected from ponds between May 3 and July 4.

THE CERCARIA

The cercaria of *Ostiolum* belongs to the ornate group of xiphidiocercariae which was created by Lühe (1909) to include all distome cercariae with a stylet and a fin on the tail. They are very small. The average length of the body of the living cercaria of *O. oxyorchis* is 0.127 mm., with a tail which is an additional 0.100 mm. in length. The body may be extended to over 0.300 mm. while under the coverglass. The width of the body is about 0.062 mm. The stylet is spear-shaped and ranges from 16μ in length in small cercariae secured by crushing the snail, to 22μ in length in cercariae which had emerged from the snail and were able to encyst in young nymphs of dragonflies (pl. 12, fig. 10). The oral sucker has a diameter of 0.030 mm., that of the acetabulum is 0.018 mm. The oral sucker is followed by a short prepharynx which opens into a pharynx with a diameter about equal to that of the acetabulum. Neither the caeca nor the excretory tubules could be seen in the living specimens because of the opacity. The excretory bladder and the large crura could be seen forming a Y at the posterior end of the body. The bladder would periodically empty itself, after which the crura would often remain filled (pl. 12, fig. 10). The cuticle is 1.7μ thick, and imbedded in it are exceedingly minute spines, which under the oil immersion (× 1700) could be seen barely projecting beyond the surface. The tail is provided with a fin which extends dorsally over the posterior third and around the end of the animal ventrally to a distance equal to approximately half the tail length.

The cercariae emerge during the afternoon and night, and swim continuously until near death, which occurs after about 24 hours. As a rule they were found swimming at all levels in the finger bowls in which the snails were kept in the laboratory.

Six experimentally infected *P. parvus* were segregated in small stender dishes 67 days after they had been fed the eggs of *O. oxyorchis*. The number of cercariae discharged by the snails were counted for 3 days. The smallest number discharged by a snail during 24 hours was 3; the largest number, 92. In 48 hours the most productive snail discharged 180 cercariae, and 10 days later during a period of 24 hours it shed 20 cercariae.

While swimming, the body of the cercaria is greatly contracted and folded ventrally, while the tail is greatly extended and lashes vigorously. The animals can make rapid progress while crawling beneath the coverglass. Both suckers are used during the process and most of the body's extension takes place between the two suckers. When the body is contracted the tail is greatly extended, and when the body is extended the tail is contracted.

Several laboratory-raised nymphs of dragonflies in their third instar and others somewhat older, presumably belonging to the same species, *Sympetrum illotum,* were placed in finger bowls with about 100 cercariae from the infected snails. The cercariae were quite indifferent to the nymphs, but the latter ate the cercariae when they came within reach. One nymph was observed to eat nine cercariae, and to chew up two more which were not eaten. If the cercariae touched the posterior part of the body of the nymph the anal aperture would close and the respiratory process would stop for a while. None of the nymphs in the third instar were observed to draw in cercariae through the anal opening, although it was commonly observed in older nymphs. Many of the nymphs of the third instar were infected after a few hours exposure to the cercariae. The largest number of cercariae taken from a nymph in the third instar was five. Sometimes cercariae that were swept into the anal opening of a nymph by respiratory movements were violently expelled again. It appeared that cercariae that approached the anal aperture from the ventral side of the nymph were most successful in attaining the branchial basket. A number of the smaller nymphs died after several hours' exposure to the cercariae, which indicates that they were over-infected.

About one hundred cercariae were drawn into a long pipette with a rubber tube over half its length. A light was directed on the other half as the pipette was placed in a horizontal position. This was examined from time to time to see if the cercariae were phototropic. So far as could be determined they showed no reaction to light or darkness.

THE METACERCARIA

The metacercariae of *O. oxyorchis* are found in nature encysted in the adults of *Sympetrum illotum* (Hagen) and in *Plathemis lydia* (Drury) in the vicinity of Oakland and Berkeley, California. The blue dragonfly, *Mesothemis* (*Erythemis*) *simplicicollis* (Say), which was very numerous at the same ponds, has been repeatedly examined but no infection has ever been found in it.

Seven nymphs collected at the Lone Tree Pond in Oakland on July 25, 1931, were infected with from one to eight metacercariae. It is assumed that the nymphs were *Sympetrum illotum* since this is the only species whose adults have been found infected at this pond, and since, also ***Plathemis lydia*** has never been observed here although the other species have been abundant.

On May 26, 1932, 26 adult *Sympetrum illotum* were examined and 12 were found to be infected with from 1 to 9 metacercariae. This and other data available indicate that about 50 per cent of this species is infected at the pond. The infection of *O. oxyorchis* in these dragonflies is very light compared to that of *O. medioplexus,* which Krull (1931) found to be about 100 per cent.

Four cysts of metacercariae taken from collected nymphs measured 0.185 mm. in diameter. One taken from a nymph three days after the infection measured 0.190 mm. The cysts ranged in diameter up to that figured, which was 0.357 mm. (pl. 12, fig. 11). Two cysts that were taken from adult *Sympetrum illotum* measured 0.245 mm. All the above measurements were made without the use of coverglasses. These differences in size of the metacercariae undoubtedly represent different stages of growth.

The flukes were usually quiet, while in the cysts, but when disturbed they moved about actively and by extending the body changed the shape of the cyst, which is normally spherical. The large Y-shaped excretory vesicle is the most distinctive feature of the adolescaria while both in and out of the cyst. The cysts differ in appearance from that figured by Krull (1931) for *O. medioplexus.* They are usually imbedded in the base of the leaf of the gills in the nymphs, sometimes rather loosely, so that they become detached while the insect is being dissected. In a nymph infected three days before, the flukes had formed cysts and the excretory bladder and its crura were just beginning to fill up with refractory granules. The stylet was also still intact in all these young cysts.

In the adult dragonflies the cysts are usually located about 1 cm. posterior to the Malpighian tubules, and cluster about the region where the respiratory organ has undergone atrophy. They are held in place by an elastic membrane, which undoubtedly was formerly associated with the branchial basket. Krull (1931) found that the cysts opened in less than an hour if placed in artificial gastric juice. This method was not used in this study, the cysts being opened, instead by pressure of a coverglass or with a needle. On being freed from the cyst the animal immediately became very active and began crawling like a measuring worm across the slide, but made little progress. Such adolescariae would live for hours in normal saline solution. The cuticle on these flukes is heavily beset with small translucent spines which are very difficult to see and usually are not to be seen at all on specimens that have been fixed and stained, doubtless because the cuticle had been sloughed off in this process.

The most conspicuous thing about the adolescaria after it is removed from the cyst is its great activity and the large Y-shaped excretory vesicle with the crura, which then are filled with refractile granules that make the organ appear almost black (pl. 12, fig. 11). While the animal was subjected to some pressure of the coverglass these refractile granules were ejected from time to time from the excretory pore. After fixation in Bouin's fluid these granules disappeared, but the walls of the organ could still be distinguished (pl. 12, fig. 14). While the animals were living a number of other features could be distinguished, which after fixation and staining could not be seen, e.g., the large glands (not shown in the figure) located just anterior to the acetabulum. The nature and distribution of the excretory tubules (fig. A) is also obscured in preparations.

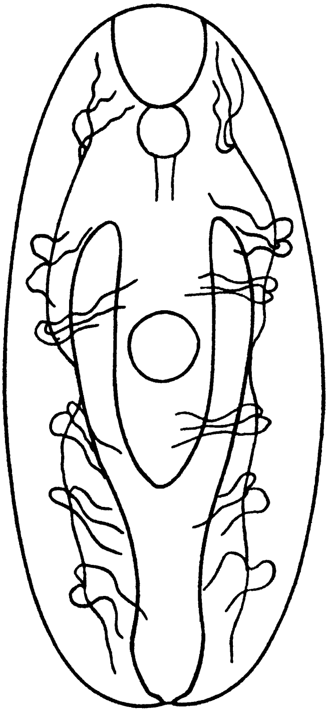

Fig. A. Diagram showing branching of the excretory system of *Ostiolum oxyorchis* (Ingles).

The excretory tubules originate from the sides of the excretory crura and run both anteriorly and posteriorly. Each of the four main branches gives off three pairs of three smaller tubules which end blindly in various regions of the body (fig. A). Although a search was made for flame cells only one near the oral sucker was ever seen, and this in one specimen only. A very short prepharynx is clearly observed if the animal is well extended. A fluke just released from the cyst was 0.685 mm. in length, and when extended, 0.801 mm. After contraction it was only 0.400 mm. long. Another fluke which was 0.566 mm. long when extended measured only 0.340 mm. when contracted.

The average length of five adolescariae just after release from the cyst and killing by gentle heat was 0.592 mm., with a maximum length of 0.767 mm. and a minimum length of 0.399 mm. Since these flukes were all taken from the same nymph it is probable that the several sizes represent different ages. The average width of these flukes was 0.239 mm. In three individuals the oral sucker averaged 0.094 mm., the acetabulum 0.066 mm., and the pharynx 0.071 mm. in width. In fixed and stained specimens the length was 0.319 mm.; the width, 0.094 mm. The oral sucker measured 0.039 mm. and the acetabulum 0.023 mm. Since these last specimens showed the genitalia clearly and probably were mature adolescariae, it seems that the fixing and staining caused considerable shrinkage.

The anlagen of the testes and ovarian complex are discernible in all the stained animals, although sometimes they are limited in size to a few cells. A very short prepharynx, a strong pharynx, and a short esophagus are present. The intestinal caeca are quite often of unequal length.

THE IMMATURE AND ADULT FLUKES

The studies of the parasite in the frog were based on both natural and experimentally induced infections. The frogs were collected from streams and pools near Berkeley and were kept in the laboratory in individual containers for a few weeks for fecal examinations. If an animal was found to be infected it was usually killed and the flukes mounted for study. If no eggs appeared in the feces after 30 to 40 days the frogs were transferred to the frog cage in the zoology pool. This cage was tightly screened to keep out insects larger than a blowfly, and was so built as to give access to water, which entered the box through a screen. Fish heads were placed in the box and served to attract flies which provided the frogs with an abundance of food. If the negative frogs showed no eggs in the feces for 40 days they were used in the feeding experiments. The feeding was accomplished by pipetting the metacercariae, which had been dissected from the nymphs, into the mouth of the frog. This was done regularly in order to secure a graded series of flukes. The feces of the frog were carefully examined every few days for eggs of the parasite. When eggs appeared in the feces the frog was killed and the flukes were studied alive for a while and then mounted for more detailed study.

It was noticed that all the adult frogs from ponds were infected. Those that came from the small streams in the deep shady canyons were never infected with *O. oxyorchis,* but the larger ones commonly were found to harbor *Haematoloechus tumidus* Ingles.

Nearly all young frogs from streams were uninfected and therefore suitable for feeding experiments. The fact that the adult frogs from the two different habitats were always infected with different flukes may be explained when the distribution of the parthenita-host or adolescaria-host is known.

The heaviest infection of *O. oxyorchis* recorded was in an adult *Rana aurora* collected from Lone Tree Pond, February 12, 1932. In this frog there were 54 mature flukes. The weight of these flukes was equal to that of the frog's lungs, it being 9 grams in each case. The heaviest infection secured in the laboratory was 34 flukes of varying sizes from maturity to those of metacercarial dimensions.

The time required for the flukes to reach maturity varied greatly in the different frogs used in the experiments. In one young *Rana aurora,* fed in July, 1931, the time was 58 days; in another, fed in April, 1932, the time was only 23 days. In *Rana boylii* after 30 days the flukes were not mature, but they were able to develop in this species. A *Rana pipiens* from the East fed metacercariae on three different occasions contained no flukes of this species when killed. Controls were always kept with the frog feeding experiments.

Only one of the 15 naturally or artificially infected frog used in this study was emaciated. This was a young *Rana boylii.* The rest were apparently quite healthy, even though the lungs were packed with flukes.

Unsuccessful attempts were made to watch the emergence of the metacercariae from the cysts placed in the opened stomach of a recently killed frog. After thirty minutes the flukes did not emerge, and when helped out with needles they merely wandered about in the open stomach and finally crawled into folds and remained there.

An infected lung of *Rana aurora* containing five mature flukes was fixed and serially sectioned to determine the method and place of feeding used by the parasites. None of the flukes was attached by the acetabulum and only two were attached by the oral sucker—in both occurrences to the respiratory epithelium in the alveoli (pl. 11, fig. 7). It is under this epithelium that the blood comes closest to the surface, since there is less connective tissue and smooth muscle. Beneath the ciliated patches of epithelium in the lumen of the lung there is a considerable mass of smooth muscle and a great deal of connective tissue (Ecker, 1889). Recently van Thiel (1930) found that *Haematoloechus variegatus* also attaches to the ciliated patches of epithelium. Since he found only erythrocytes in the gut of the fluke he supposed that the different sizes of flukes of the same age could be accounted for by these differences in the place of attachment; that is, the flukes attached to the ciliated patches would be smaller because they would get less food than those attached to the respiratory epithelium. During this study, however, ciliated cells were repeatedly observed swimming about in the caeca of living trematodes. These were at first thought to be parasitic ciliates, but when stained they were found to be epithelial cells that had become rounded after detachment from the frog's lung. There were many places in the serial sections where the epithelium was destroyed, doubtless the former pasturing places of the flukes. There is little doubt that the ciliated epithelium, tesselated respiratory epithelium, connective tissue, blood cells, and perhaps also smooth muscle go to make up the solid food diet of *O. oxyorchis.* A section of the lung (pl. 11, fig. 7) shows the respiratory epithelium destroyed and the blood cells with mucus deep in the oral sucker, with connective tissue and muscle cells being sucked in from the injured wall of the alveoli. Travassos and Darriba (1930) show a photograph of a section of a lung of *Leptodactylus ocellatus* in which *H. neivai* is attached to the respiratory epithelium.

The anlage of the genitalia is discernible even in the mature cercaria, where the oblique testes are only a little larger than the surrounding nuclei. The female genitalia in the cercaria are merely a mass of cells containing large nuclei dorsal and slightly posterior to the acetabulum. In the mature cercaria the testes are composed of many cells and the female genitalia assume the shape of an inverted Y the base of which now extends somewhat anterior to the acetabulum and then is reflexed back toward it (pl. 12, fig. 14). As the young fluke grows this portion of the genitalia, which is anterior to the acetabulum, differentiates and becomes the cirrus sac and the metraterm, while the part posterior to the acetabulum becomes the ovary and possibly the seminal receptacle (pl. 12, fig. 7). As the fluke grows the genitalia anterior to the acetabulum are likewise elongated so that the genital opening is always in the fork of the intestinal caeca. The uterus first appears when the flukes are nearly sexually mature as a more or less coiled tube extending to the posterior end and back to the genital pore. Whether it is formed *in situ* or by growth and elongation of the parts of the genitalia that were present in the metacercaria could not be determined from the material. There is a lumen in it when it first appears.

The vitellaria begin to form anteriorly and differentiate posteriorly as the animal becomes mature. These glands are the last part of the reproductive system to form. The ovary is at first round or oval and later elongates until it is about three times as long as wide.

Another change which is quite pronounced as the fluke grows to maturity is the changing position of the acetabulum. In the cercaria, metacercaria, and the young fluke it is in the posterior half of the body, but its position gradually approaches the anterior half, as the posterior part of the animal grows faster than does the anterior one. When full maturity is reached it lies in the first third of the body (pl. 11, fig. 3).

It was noticed that flukes which had recently reached maturity in the feeding experiments were strikingly different from those that had been in the frogs during the hibernation period. These two forms were described as separate species (Ingles, 1932) and their identity was not suspected until this life-history was followed through. In the recently matured adult fluke the structures are those described for *O. confusus* (Ingles, 1932), while in the older form the structures are those described for *O. oxyorchis* (Ingles, 1932).

The latter name will be retained because it was first used, and *O. confusus* becomes a synonym.

The young adult is covered with small spines and the cuticle bearing them is not shed during fixation, while in the old flukes the cuticle is often lacking or in patches before fixation. After fixation the cuticle is invariably sloughed off and never appears in the permanent mounts. The pharynx and the oral sucker are much larger in proportion to the body width in the young adult than they are in the older fluke. Also, the ovary and testes are often deeply incised in the young adults, while in the older form they are oval and smooth, and the ovary is elongate. These are a few of the more important changes which the flukes undergo even after maturity. A detailed description of the adult has been given elsewhere (Ingles, 1932).

FINAL LIFE-HISTORY EXPERIMENT

The eggs of *O. oxyorchis,* which were laid by a mature fluke removed from an adult *Rana aurora* on May 9, 1932, were sprinkled on laboratory-raised algae and soaked willow leaves. The algae and leaves were placed in a gallon jar filled with water and in this were placed several young laboratory-raised *Planorbis parvus* on May 16, 1932. This jar was covered and placed outside in a pool to maintain a constant temperature. Two of the snails were examined on July 13 and both were found to contain sporocysts with immature cercariae. On July 18 another snail was examined and several of the cercariae were mature enough to swim. Six out of ten of the segregated snails proved to be infected, and cercariae began to emerge on July 19, 1932. One of the snails did not shed cercariae until July 23. The control snails showed no infection.

Ten laboratory-raised nymphs of the species *Sympetrum illotum* which had hatched July 21, 1932, were exposed to the above mentioned cercariae for two nights and two days. One nymph was immediately killed and five small metacercariae were found in cysts with their stylets intact. Only a few granules in the excretory system were visible. Three of the nymphs died after two days. They were in the third instar. Some of the metacercariae from the above mentioned nymphs were dissected out and fed to two young *Rana aurora* which had only recently metamorphosed. These frogs were not laboratory-raised, but had been collected just after they had metamorphosed. They were kept under laboratory conditions for over a month. During this time no eggs appeared in the feces. One of these frogs was killed 35 days after the first feeding. The largest of the flukes in it were not quite mature. Eggs appeared in the feces of a similar frog 48 days after the first feeding. This and other similar experiments indicate that the frogs reach maturity in a little over 40 days.

DISCUSSION

In order to appreciate the relationships of the parthenita hosts of those frog lung flukes whose life-histories are already known, one must be familiar with the opinions of conchologists on the classification of the Planorbidae. Walker (1918) considers the genus *Planorbis* to be composed of six subgenera of which *Gyraulus* is one. Other authorities raise these subgenera to generic rank, but in this paper the name *Gyraulus* is used as a subgenus of *Planorbis.* Krull (1931), in his work on *Ostiolum medioplexus* and *Haematoloechus parviplexus,* treats *Gyraulus* as a genus. These differences in the use of a generic or subgeneric name depend on the investigator who identified the hosts, and must be understood in considering the host specificities and host relationships in the frog lung flukes.

Some uncertainty also exists concerning the systematic status of the California snail identified by Dr. G. D. Hanna for the writer as *Planorbis* (*Gyraulus*) *parvus* Say. Dr. Hanna advises the writer that Mr. Frank Baker considers the western species to be *Planorbis* (*G.*) *similaris* (Baker), but since Dr. Hanna was unable to "see the slightest difference" between the western species and eastern *Planorbis parvus,* he gave the latter name to the California snail in his identification of it and this name is used in this paper.

If, among the frog lung flukes, there is a rigid specificity of each species for a definite parthenita host, one would expect to find that closely related flukes have closely related snails as parthenita hosts. In the investigations of the known life-histories of three North American frog lung flukes this expectation is not realized. The California species of *Ostiolum oxyorchis* has as its natural host *Planorbis parvus,* which is the same host, according to some authorities, as that which harbors *Haematoloechus parviplexus* in Michigan, while the Michigan species, *Ostiolum medioplexus,* requires for its host a species in another genus, *Segmentina* (*Planorbula*) *armiger.* In view of these facts one is forced to question either the validity of *Ostiolum* as a separate genus from *Haematoloechus,* or the specificity of the frog lung flukes for a definite species of snail host, or the classification of the Planorbidae. The data are too meager to justify conclusion, but they indicate that the frog lung flukes require a species of planorbid snail for their best development, and that at least one species, *Ostiolum oxyorchis,* may also develop in another closely related species of snail.

The degree of specificity for the parthenita host shown by trematodes varies with the group to which the parasite belongs. Sinitsin (1931) found seven species of Harmostominae, each of which was rigidly specific for one snail host. These observations were based only on natural infections as they occurred in over one thousand snails examined from the vicinity of Washington, D. C. Faust (1929) states that seven species of *Melania* and possibly also the genus *Ampullaria* may harbor the cercariae and parthenitae of *Paragonimus westermani* (Kebert, 1878). Faust and Nishigori (1926) found that two species of *Monorchotrema* have as parthenita hosts two species of *Melania. Fasciolopsis buski* (Lankester) is known to develop in species both of *Planorbis* and *Segmentina* (Faust, 1929). This list might be enlarged with examples from other groups, but enough have been given to show that the specificity of the trematodes for the parthenita hosts varies greatly and that it depends to a great extent on the family or subfamily to which the parasite belongs.

When a comparison is made of the life-histories of *Ostiolum oxyorchis* and *O. medioplexus* many differences are at once evident in the structure of the different stages. Unfortunately, Krull (1931) found the life-histories of *O. medioplexus* and *Haematoloechus parviplexus* so similar that he neglected to figure all the stages of the latter species, so that a complete comparison of the life-history stages can be made only between the Michigan and California species of *Ostiolum.*

The eggs of *Ostiolum oxyorchis* probably could not be distinguished from those of *O. medioplexus,* since the size and shape are nearly the same for both.

The miracidia seem to differ considerably. They are longer and narrower in *O. axyorchis* than in *O. medioplexus.* The epidermal plates are scarcely or not at all discernible in the California miracidia, while they appear prominently in the figures of Krull (1931) for the Michigan species. The number of nuclei are found to be constant in *O. oxyorchis,* but variable in *O. medioplexus.* The flame cells were seen and figured by Krull (1931) for the eastern species, but could not be seen in *O. oxyorchis.* The asymmetry of the body of the miracidium which was characteristic of *O. medioplexus* was observed only once in *O. oxyorchis.* The above mentioned features show the miracidia of the two species to be quite distinct. The evidence at hand indicates that both of the miracidia bore through the intestinal wall of the parthenita host.

From the data secured during this study and also in that of Krull's (1931), it could not be determined whether the miracidia develop directly into sporocysts, which produce cercariae directly, or whether a second generation of sporocysts is developed from the metamorphosed miracidia. Sinitsin (1931) is inclined to believe that those flukes whose eggs must be ingested by the snail in order to liberate the miracidia do not produce a daughter generation of sporocysts and that the cercariae develop in the "founder" or "mother" sporocysts. He believes that the miracidia, which emerge from the eggs ingested by the snail, bore directly through the snail's gut into the liver, while those miracidia that have a free-swimming life enter other tissues less nutritious and produce an erratic generation of parthenitae which migrate to other tissues. A few of these latter sporocysts finally reach the liver of the snail and continue to develop and produce cercariae. It is suggested that *Ostiolum oxyorchis* would lend itself favorably as material for a study of the sporocysts and their generations since the nuclei in the miracidia are definite in number and kind. These nuclei could be easily differentiated in the tissues of the snail if the proper cytological procedure were followed. The shape of the sporocyst of *O. oxyorchis* does not seem to differ materially from that described by Krull (1931). Sporocysts are found to contain cercariae in nearly all stages of development in a snail that has just started shedding cercariae.

The cercariae of *Ostiolum oxyorchis* are very similar in size and structure to those described by Krull (1931). The anlagen of the reproductive system and the intestinal caeca could be distinguished only in the stained specimens and are therefore not shown in the figured specimen, since it was drawn from an unmounted cercaria (pl. 12, fig. 10). The time required for the parthenitae to produce cercariae is the same in *Ostiolum oxyorchis* and *O. medioplexus*. The cercariae of *O. medioplexus* emerge at night while those of *Haematoloechus parviplexus* emerge during the afternoon. The cercariae of *O. oxyorchis* emerge during both afternoon and night. The method of entering the dragonfly host seems to be the same for the cercariae of all three above mentioned species.

A considerable degree of specificity is manifested for the adolescaria host by the frog lung flukes. In all the species known this host is an insect. *Haematoloechus variegatus* (Rudolphi) in Europe has recently been shown to use the larvae of *Anopheles maculipennis* for this host (van Thiel, 1930). In the United States Krull (1931) found that two species of *Sympetrum* were used by *O. medioplexus* and *Haematoloechus parviplexus* and that other closely related dragonflies could not be infected. In *O. oxyorchis* most of the natural infections occurred in a pond-inhabiting dragonfly, *Sympetrum illotum* (Hagen). The infection, however, was found twice in a closely related genus, *Plathemis lydia* (Drury). It was not found in another genus, *Mesothemis simplicicollis,* which belongs in the same subfamily as do the infected species. Only the nymphs of *Sympetrum illotum* were infected experimentally. Experimental feeding with other species was not attempted. Since the uninfected green-eyed nymphs of *Mesothemis simplicicollis* were often taken in the same sweep of the net with infected nymphs, which were presumably those of *Sympetrum illotum,* it seems that differences in habitat must be excluded as an explanation of the lack of infection in *Mesothemis simplicicollis.* Mechanical factors, possibly, such as inhibiting structures of the anal aperture or branchial basket, may account for the lack of infection in *Mesothemis simplicicollis,* but the biochemical complex of the nymph is perhaps a better explanation.

The presence of the infection by *Ostiolum oxyorchis* in frogs collected from ponds, and its absence in frogs of the same species collected from small streams, is significant. *Haematoloechus tumidus* was often found in the stream-inhabiting frogs, but never in frogs collected from ponds. The life-cycle of *H. tumidus* is not known, but its presence in *Rana aurora* which inhabit streams both near Berkeley and near Bakersfield, California, suggests that its adolescaria host will be found to be a stream-inhabiting dragonfly or other insect. This supposition is strengthened by the fact that the Bakersfield stream containing the infected frogs was rich in pulmonates, especially planorbids. Needham and Heywood (1929) report a species of dragonfly belonging to the genus *Libellula,* which in the eastern United States is found only about small streams and rarely about ponds. It is quite possible that such a dragonfly is present on the west coast and therefore may be the adolescaria host of *H. tumidus.* This would explain the fact that *Rana aurora* in streams harbored only *H. tumidus,* while the same species of frog from ponds was found to be infected only with *Ostiolum oxyorchis.*

SUMMARY

The eastern frog, *Rana pipiens,* could not be infected with *Ostiolum oxyorchis. R. boylii* could be infected experimentally, but was not found harboring the flukes in nature. *R. aurora,* which inhabits ponds but not streams was found infected in nature.

The adolescaria hosts for *O. oxyorchis* are the dragonflies, *Sympetrum illotum* (Hagen) and *Plathemis lydia* (Drury). The nymphs of *Mesothemis simplicicollis* Say were not infected in the pond where 50 per cent of the *S. illotum* harbored metacercariae

Planorbis (*Gyraulus*) *parvus* Say and *P.* (*G.*) *vermicularis* Gould were experimentally infected by feeding them with eggs of the fluke. The infection was found only in the former species in nature. The parasite feeds on nearly all the tissues in the lung of the frog.

The average time required for the development of the parasite in the parthenita host is between 60 and 70 days.

The time required for development in the frog varies, but most of the experiments indicate it to be about 40 days.

O. oxyorchis shows considerable specificity for its hosts. *Ostiolum confusus* (Ingles) is found to be a synonym of *O. oxyorchis.* Profound changes in the structure of the adults, which vary with successive seasonal growth, account for the previous separation of these forms as different species. The study of the life-history shows them to be the same species.

The parthenita hosts of *O. oxyorchis* are more closely related to those of *Haematoloechus parviplexus* (Irwin) than they are to those of *O. medioplexus* (Stafford), which indicates that the genus *Ostiolum* may not be a natural one. Other factors, however, such as an unnatural classification of the

Planorbidae, might account for the difference in the relationship of their hosts.

The study of life-histories with reference to host specificities is a valuable aid in problems concerning the evolution of both the parasite and its hosts. The investigator of the present and former distribution of the hosts and parasites, and of the adaptations of adventitious animals, might likewise find this method of study valuable.

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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida.

PLATE 11

Ostiolum oxyorchis

Fig. 1. Miracidium, fixed in Schaudinn's fluid, stained with Heidenhain's haematoxylin. × 1650.

Fig. 2. Young fluke just before maturity, ventral view, × 53.

Fig. 3. Mature fluke, ventral view. × 29.

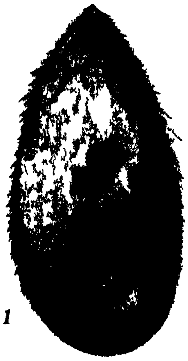
Fig. 4. Miracidium in the wall of the gut of *Planorbis parvus.* × 850.

Fig. 5. Egg. × 850.

Fig. 6. Miracidium emerging from the egg. × 850.

Fig. 7. Section of a portion of a frog's lung showing parasite attached. × 53.

Fig. 8. Living miracidium. × 850.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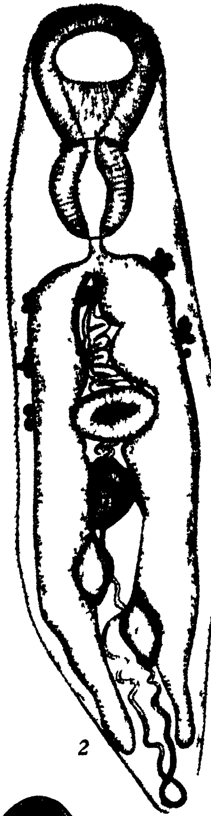
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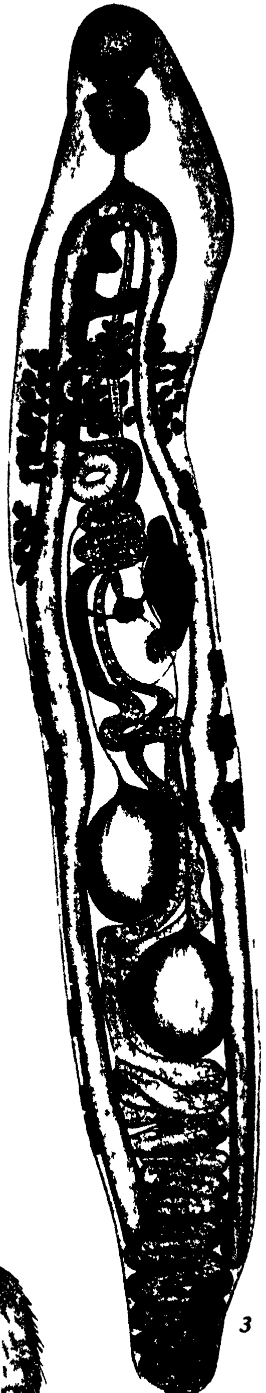
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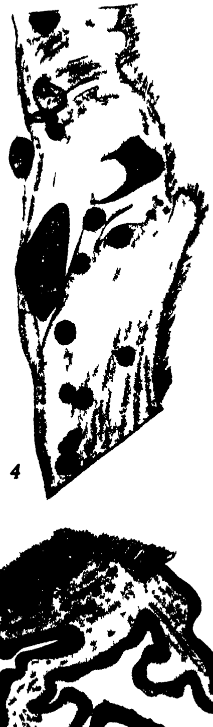
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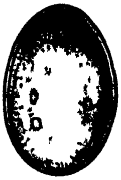
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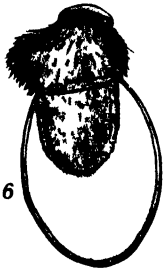
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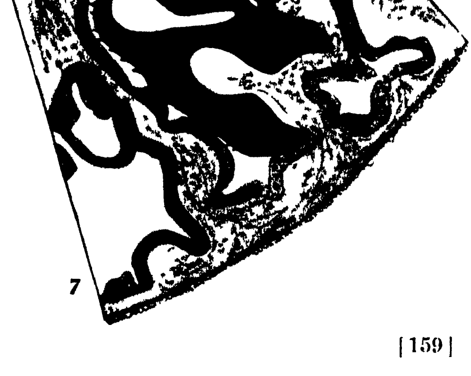
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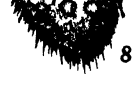
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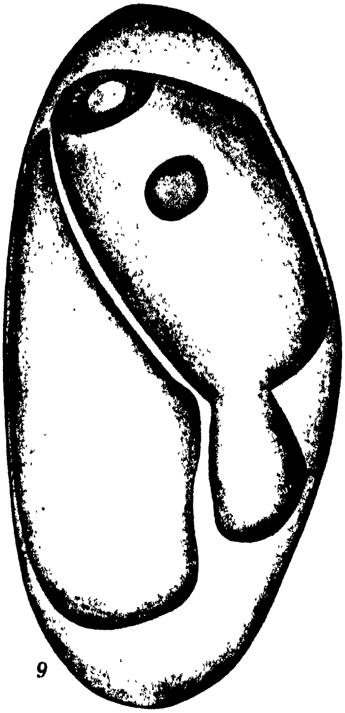
PLATE 12

Ostiolum oxyorchis

Fig. 9. Sporocyst. × 385.
Fig. 10. Cercaria, drawn from life. × 385.
Fig. 11. Metacercaria, dissected from a nymph. × 235.
Figs. 12 and 13. Stylets of cercariae. × 385.
Fig. 14. Metacercaria removed from cyst, stained, ventral view. × 235.
Figs. 15 and 16. Young sporocysts with germ balls. × 385.
Fig. 17. Young fluke. × 235.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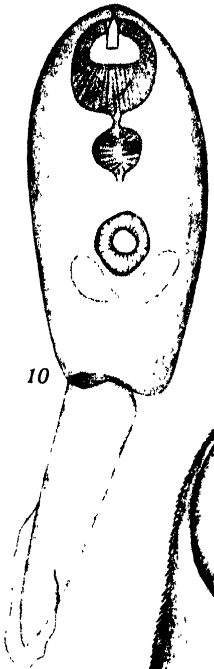
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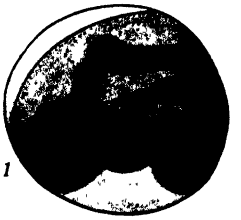
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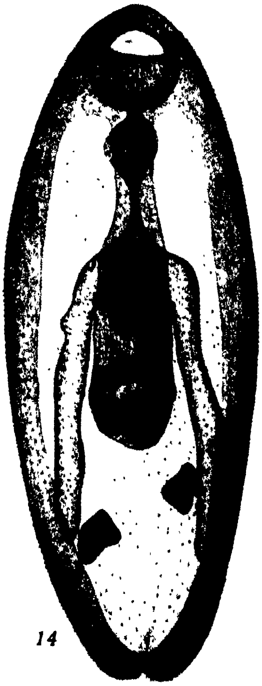
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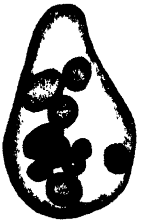
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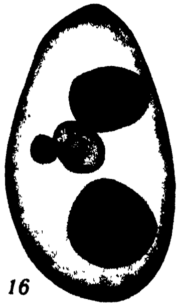
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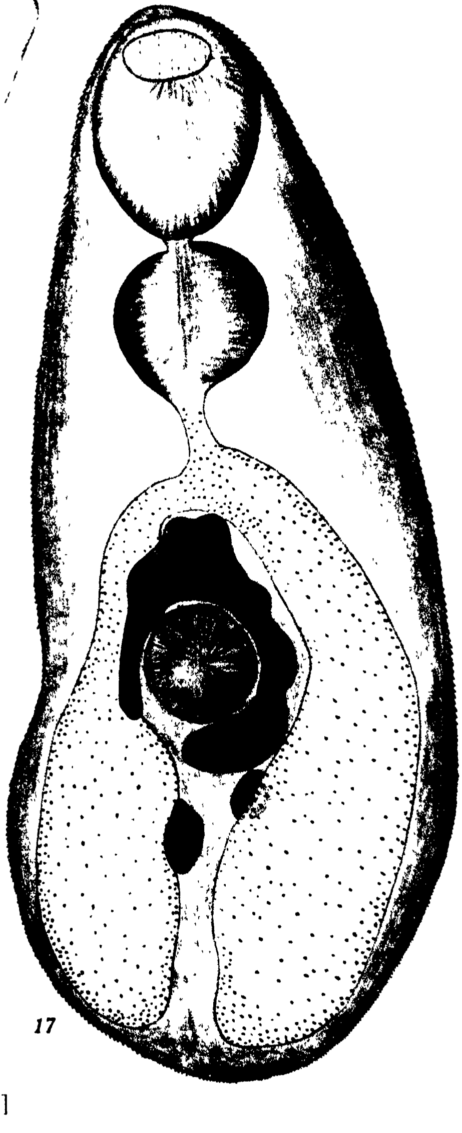
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STUDIES ON THE STRUCTURE AND LIFE-HISTORY OF ZEUGORCHIS SYNTOMENTERA SUMWALT, A TREMATODE FROM THE SNAKE THAMNOPHIS ORDINOIDES FROM CALIFORNIA

BY

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STUDIES ON THE STRUCTURE AND LIFE-HISTORY OF ZEUGORCHIS SYNTOMENTERA SUMWALT, A TREMATODE FROM THE SNAKE THAMNOPHIS ORDINOIDES FROM CALIFORNIA

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INTRODUCTION

The present paper deals with the structure and life-history of *Zeugorchis syntomentera* Sumwalt from the western garter snake, *Thamnophis ordinoides.* It appears to be the first work on the life-history of either of the species of *Zeugorchis,* although McCoy (1928) gives notes on the larval forms of three genera belonging to the same subfamily. The life-history of this species has not been demonstrated with laboratory-raised material, but the stages and hosts involved in the cycle are known.

The snakes were identified by Dr. Jean M. Linsdale, and Dr. G. D. Hanna identified the snail host.

ACCOUNT OF ZEUGORCHIS SYNTOMENTERA SUMWALT

HISTORICAL ACCOUNT

Stafford (1905) described a fluke from the esophagus of the eastern Canadian garter snake *Thamnophis sirtalis* (L.), which he called *Zeugorchis aequatus* because of the symmetrically paired testes. This was the first use of the name *Zeugorchis* for a genus of animals; consequently *Z. aequatus* is the type species of the genus. Unfortunately, Stafford did not publish a figure and his description is very brief.

Later, Nicoll (1906) used the same generic name for a fluke which he found in the *bursa fabricii* and cloaca of the herring gull *Larus argentatus.* This fluke, *Zeugorchis acanthus,* is so different from the one described by Stafford (1905) that it certainly cannot remain in the same genus. It differs from *Z. aequatus* in almost every important feature and apparently belongs to the Echinostomidae, and not, as does Stafford's *Z. aequatus,* to the Plagiorchiidae. Because of priority, it is evident that *Zeugorchis* must be retained for Stafford's fluke from the garter snake and rejected for the fluke described by Nicoll (1906) from the herring gull.

Stafford (1905) expressed the opinion that *Distomum boscii* Cobbold and *Zeugorchis aequatus* belong to the same genus. Cobbold's description (1859) is very brief and inadequate, and his figure does not indicate much similarity to *Zeugorchis aequatus.* Most of the structural peculiarities of Stafford's species of *Zeugorchis* are not found in *D. boscii,* and this latter species therefore cannot be included in the genus *Zeugorchis.*

Sumwalt (1926) described a fluke which, though it differs slightly from Stafford's species, *Zeugorchis aequatus,* undoubtedly belongs to the same genus. This fluke, which she named *Zeugorchis syntomentera,* she found in great numbers in the mouth and esophagus of *Thamnophis sirtalis* and *T. ordinoides* from the state of Washington.

The flukes which the writer found in the alimentary tracts of *Thamnophis ordinoides* from California are indistinguishable from *Zeugorchis syntomentera* and will therefore be considered as belonging to that species.

Sumwalt (1926) believes that *Mediorima propria* Nicoll (1914) also belongs to the genus *Zeugorchis,* but there are many differences which exclude it from this genus as defined by Stafford (1905). *Mediorima propria* Nicoll was, however, found in an American snake belonging to the genus *Natrix.* The generic and specific characters of the fluke were defined on one specimen. Thus the material as well as the description are inadequate to determine whether or not *Mediorima propria* should be placed in the genus *Zeugorchis.* Because of the many fundamental points of difference between *Mediorima propria* and *Zeugorchis aequatus* the writer believes that *Mediorima* should be retained for Nicoll's fluke.

In this paper *Zeugorchis* will be considered as belonging to the Plagiorchiidae and the subfamily Reniferinae as defined by Pratt (1903). At present there are but two described species in the genus, both of which occur in the alimentary tracts of garter snakes in North America, namely, *Zeugorchis aequatus* from *Thamnophis sirtalis* of eastern Canada, and *Z. syntomentera* from *T. sirtalis* and *T. ordinoides* from the western United States.

Until the present work was done, nothing was known concerning the life-histories of either species of this genus. Although the life-history has not been entirely established by the experimental feeding of laboratory-raised animals, all the stages of the life history are known. Over fifty specimens of *Thamnophis ordinoides* from northern and central California were collected and examined during this investigation, which extended over a period of three years. The number of infections of *Z. syntomentera* are rare compared to the number reported by Sumwalt for the same species of snake from the state of Washington. No other species of snake fluke was found in the California *Thamnophis ordinoides.*

GENERAL METHODS

The study of the life-history of *Zeugorchis syntomentera* was begun when it was discovered that certain xiphidiocercariae from *Physa gyrina* penetrated and formed cysts in the tail and body of the tadpoles of *Hyla regilla.* These encysted adolescariae were dissected out and fed to young chickens, mice, and several vertebrates, but they failed to develop in them. They were also fed, however, to young garter snakes, *Thamnophis ordinoides,* which were afterward killed at various times, and it was observed that the adolescariae had continued to develop in the alimentary tract. The young snakes that were used in the experiments were collected in the vicinity of Berkeley, California, during June and July, 1929, and had apparently not yet acquired the infection since it was never found in the control snakes but was always present in those which had been fed the infected tadpoles.

Although the writer has examined fresh-water molluscs from various parts of California for larval trematodes, he found the cercariae concerned with this infection only in one place, and there only during the summer of 1929. They were found in *Physa gyrina* from an artificial reservoir in Oakland, California.

The infected snails which were collected from this reservoir during the first part of July shed cercariae when placed in a small watch glass in the laboratory. The cercariae were studied alive under a coverglass. To prevent curling they were held in an extended position by placing a rubber band about both the slide and coverglass and plunging them into hot Bouin's fluid. The cercariae were later removed from the slide with a small camel's hair brush and placed in small stender dishes for staining.

The adolescariae were dissected out of the cyst and treated in much the same way as were the cercariae. The young flukes were secured by feeding the infected tadpoles to the marita hosts and killing these at various times after the infective meal. Like the cercariae and the metacercariae, these young flukes were studied alive and then fixed and stained for permanent mounts.

An attempt was made to secure adult flukes by feeding two young garter snakes on infected tadpoles, but both snakes died before adults could be secured. A search was then made for naturally infected snakes in order to start the infection in laboratory-raised animals and to secure the adults, the eggs, and the miracidia. The last mentioned stage was not then known. Until the summer of 1932 only the immature forms had been found, but in July of that year two large garter snakes were found with the adults of *Zeugorchis syntomentera.* In one of these snakes there were also immature flukes, representing many stages, which were undoubtedly also of the same species. Eggs were extruded when the adults were put in cold distilled water. The eggs did not hatch in pond water after a period of several weeks in the laboratory. They did hatch, however, even just after they were laid, when they were fed to snails.

The miracidia were obtained for study by placing young *Physa gyrina* in a watch glass with the eggs of the parasite for several hours, then isolating the snail in a clean watch glass and watching it closely for defecation. The feces were quickly transferred to a slide, crushed with a coverglass, and examined under the microscope for miracidia.

Two attempts were made to infect snails belonging to *Physa gyrina* Say and *Planorbis* (*Gyraulus*) *vermicularis* Gould by feeding them the eggs of the parasite. After 72 days no infection had been observed, and the experiment was discontinued for lack of material.

LIFE-HISTORY

THE EGG

The eggs of *Zeugorchis syntomentera* are oval in outline and light brown in color. They vary in length from 46μ to 53μ, and ten of them averaged 48μ in length by 24μ in diameter. These measurements were made in distilled water just after the eggs were laid. Internally, the outlines of the miracidium can readily be distinguished. In many of the eggs are fine granules which lie between the miracidium and the shell and may represent the remains of yolk cells. While the eggs are still unhatched some heavy lines can be seen on the anterior end of the miracidium. Another internal feature which is characteristic of the unhatched egg is the large globule of clear substance which usually lies near the posterior end (pl. 13, fig. 5).

THE MIRACIDIUM

The miracidium is fully formed when the egg is laid. No vital movement can be seen in the egg until it has first passed through the digestive tract of *Physa gyrina,* when the movement of cilia and other slight movements can be detected. The miracidium swims very sluggishly and continues to extend and contract itself during the process. It can attach itself to the coverglass with its small star-shaped sucker at the anterior end. It is elongated with the widest part in the first half of the body. The cuticle of the miracidium is very thick and the entire body is covered with cilia except the extreme posterior end, which has none or only a few stubs. Not much of the internal structure can be distinguished when the specimen is in the natural condition, but when the miracidia are stained with various intra-vitam dyes, especially methylene blue, two rather large nuclei can be seen in the anterior half of the body and two somewhat heavier and still larger ones in the posterior part (pl. 13, fig. 3).

Since the eggs can be induced to hatch only by feeding them to snails, and since the miracidia live only for a short time in the water, it is assumed that the latter do not have a free-swimming period but bore directly through the wall of the snail's gut and become sporocysts.

THE SPOROCYST

Several snails, *Physa gyrina,* were dissected from time to time to secure the parthenitae. No rediae were ever observed. Only the very young sporocysts could be successfully dissected out of the snail's liver. One of these sporocysts measured 0.446 mm. in length and contained three germ balls, which probably represent developing cercariae (pl. 13, fig. 2). This sporocyst was twisted at the anterior end; the posterior end was narrower than the rest of the body and was somewhat club-shaped. No signs of sporocysts could be found in the liver of *Physa gyrina* 72 days after feeding to the snails the eggs of the parasite.

Some attempt was made to study the percentage of infection of the *Physa gyrina* from the reservoir near the Dimond Boy Scout Camp in Oakland, California. One hundred and sixty *Physa gyrina* were examined between July 5 and July 25, 1929. The percentage of infection was found to be slightly less than 10 per cent of all the individuals examined. Many of the snails that were opened in the first part of July contained cercariae which were too immature to swim.

THE CERCARIA

The cercariae used in this study were secured after they had emerged from the *Physa gyrina* that were brought into the laboratory. The best results were secured by studying them while they were still alive, although some of the systems were best observed in the permanently mounted material. The cercariae killed by heat slowly applied to the slide, and then fixed in hot Bouin's fluid, were found to average 0.298 mm. in length and 0.107 mm. in width. The greatest extension noted in this material was 0.316 mm.; the shortest, 0.234 mm. in length. The greatest extension noted in life was 0.410 mm., the shortest, 0.235 mm.

The tail is inserted ventrally and is capable of great extension and contraction. In eight specimens it averaged 0.220 mm. and was 0.268 mm. in the longest one that was measured. The oral sucker is subterminal and averages 0.102 mm. in diameter. In its dorsal surface, and projecting slightly from the oral sucker, it has a quill-shaped stylet, which measures 0.020 mm. to 0.024 mm. in length and 0.005 mm. to 0.007 mm. in width (pl. 13, fig. 8). In living specimens the oral sucker measures 0.067 mm. to 0.071 mm. in diameter. The acetabulum is slightly larger and averages 0.103 mm. in diameter in the preserved material. It is an important organ in crawling. It can be projected for a considerable distance beyond the ventral surface.

The oral sucker opens into a short prepharynx, and this into a pharynx which measures in living material from 0.031 mm. to 0.037 mm. in diameter. The prepharynx is 0.028 mm. to 0.032 mm. in length and 0.005 mm. in diameter. Leading from the pharynx is an esophagus which measures 0.070 mm. in length and 0.0028 mm. in diameter. The caeca are very short and their ends do not reach the anterior margin of the acetabulum (pl. 13, figs. 1 and 4).

On either side of the caeca are eight to ten cephalic glands. These open by ducts into the oral cavity. They contain an opaque substance and their shape is largely determined by the state of contraction or extension of the cercariae. Spines are quite prominent anteriorly and disappear entirely about the middle of the body.

The bladder is large and is crenate in shape. While under the coverglass it is seen to fill and rapidly contract. Anteriorly the bladder gives rise to two large crura which extend at least to the level of the anterior margin of the acetabulum. From the outer edges of each crus a small tubule arises and runs posteriorly, where it ends in three fine branches (pl. 13, fig. 4). Because of the opaqueness of the cystogenous and stylet glands the finer branches of the excretory system could not be distinguished anteriorly. There are small clear concretions in the bladder which are visible only in the living specimens.

The anlage of the reproductive system can be distinguished in stained specimens (pl. 13, figs. 1 and 7). It forms a hood-like cover over the acetabulum, forks posteriorly, and is enlarged anteriorly. The beginning of the testes can be seen behind the acetabulum. The anlage of the Laurer's canal can be seen in stained specimens when they are viewed from the side (not shown in figures).

When inactive the cercaria curls up until the posterior and anterior ends meet ventrally, and in this position it rests on its dorsal surface. When the cercaria crawls, the tail is lashed from side to side as the body is contracted, but remains quiet when the body is extended. While swimming it bends the anterior end ventrally and sometimes attaches the oral sucker to the posterior end. The cercariae show no particular response to light, but are attracted to the bodies of *Hyla* and *Rana* tadpoles.

Several experiments were conducted with these cercariae to learn something concerning their relation to the hosts of the adolescariae. One of the experiments will illustrate the method and results.

Two *Hyla regilla* tadpoles and about sixty cercariae were placed in a small dish of water on July 17, 1929. The tadpoles were in different stages of development, one having only hind legs, the other all four legs. Two other tadpoles were similarly placed, without accompanying cercariae, for controls. The control animals were quiet, while the animals exposed to the cercariae lashed about violently in the dish. After fifteen minutes no cercariae were found in the dish. They were observed to attack the tail of the two-legged tadpole, particularly on the ventral fin, by releasing the acetabulum and permitting the body to swing freely in the water, remaining attached only by the oral sucker. Peristaltic movements were observed flowing from the anterior to the posterior part of the body of the cercaria. At the same time the body of the cercaria rotated to and fro on its long axis. The anterior end of the animal was soon submerged in the tissue, where it shaped itself into a spheroidal boring organ. During this boring process the tail of the cercaria was quiescent and strongly contracted, and coiled against the body. When the animal disappeared into the body of the host the tail was sheared off at the opening. Five flukes were observed for some time moving about in the tissue of the tadpole's tail. They seemed to be going toward the tail muscle, but occasionally they turned away from it. At times the capillaries of the tail were entirely clogged as the flukes crossed them in their wanderings. After the fluke crossed such a blood vessel the tunnel formed by the fluke became filled with blood. The boring into the tadpole's tail was usually completed in five minutes, although it was observed to be accomplished in three minutes by one cercaria.

Other and similar experiments gave like results. It was found that the cercariae were attracted to mature *Hyla regilla* when the foot of one was held in the water containing them, but they could not penetrate it. They attacked and encysted in the larvae of *Rana aurora* and of the newt *Triturus torosus,* although they did not attack the adults of the latter species.

THE CYST OR METACERCARIA

The cercaria upon entering the tissues of the tadpole formed a cyst after about an hour, during which time the fluke could be seen moving about. After approximately two hours the concretions in the bladder of the parasite became dark and were plainly visible through the cyst. By careful measurements of the cysts in one tadpole's tail it was found that they continued to grow in size. The cysts in the tail measured 0.254 mm. in diameter for an average of six, after the tadpole had been infected eight days. Ten days after infection these cysts (eight measured) averaged 0.265 mm. in diameter for the same tadpole. After fifteen days twelve cysts in the same animal averaged 0.272 mm. in diameter. Further measurements were impossible owing to the metamorphosis of the tadpole.

When *Thamnophis ordinoides* were fed these infected tadpoles they became infected with the young flukes. The walls of all the cysts were digested and the flukes were free in the gut of *Thamnophis* after ten days, and evidence seems to point to a quicker consummation of this process. The average length of seven flukes fourteen days after ingestion was 0.472 mm. The greatest extension noted among these flukes was 0.609 mm. Young garter snakes which had been fed cysts and killed at various times showed young flukes identical with those freed from cysts from the tadpole's tail. The control snakes used in these experiments were always negative, although some of them were collected from the vicinity of the pond which harbored the infected snails. All the snakes, however, were usually quite young and had not yet acquired an infection.

The Young Fluke

The young flukes measured (average for ten flukes) 0.534 mm. in length and 0.200 mm. in width after they had been in the alimentary tract of the snake for five days. These measurements were taken after the flukes had been killed with hot Bouin's fluid. One of the flukes in life, greatly extended under a coverglass, measured 1.21 mm. Ten flukes that had been in the alimentary canal of a snake for nine days measured 0.350 mm. in length when killed with hot Bouin's fluid. The greatest extension in life was 1.00 mm.

In a living fluke that measured 0.672 mm. in length and 0.386 mm. in width, the oral sucker measured 0.119 mm. in diameter, and the acetabulum 0.113 mm. The length of six living flukes averaged 0.680 mm., while the same flukes measured 0.534 mm. after being killed with hot Bouin's fluid.

The young flukes differed from the cercariae in several important features. The tail was lost when the cercaria entered the tissues of the tadpole. The stylet disappeared, and the cephalic glands atrophied and dwindled almost to the point of disappearing. More of the excretory system could be distinguished in the young flukes than in the cercariae, owing to the lack of substance in the cystogenous cells. In addition to the branch that runs posteriorly on each side of the crura there are also two that run forward, one on each side. All these branch again as shown in the figure (pl. 13, fig. 9). Although a careful search was made for flame cells none was ever seen. The oesophagus and the intestinal caeca were greatly distended by some unknown substance. The acetabulum was somewhat smaller than the oral sucker. The reproductive system was scarcely more differentiated than in the cercaria.

Two young *Thamnophis ordinoides* were fed infected *Hyla regilla,* the intention being to keep the snakes until the flukes became mature. It became necessary for the writer to leave the cool August climate of Berkeley and go to the hot climate of Bakersfield, California. Although great care was exercised in transporting the snakes, both died *en route.* One was autopsied and found to harbor forty-seven immature *Zeugorchis.* These were subsequently mounted on slides. These young flukes were indistinguishable from the young flukes taken from the gut of a captured but not artificially infected *Thamnophis ordinoides,* which also harbored adult *Z. syntomentera.*

THE ADULT FLUKE

The adult specimens of *Zeugorchis syntomentera* used in this study were not raised experimentally, but were found in the mouth and oesophagus of two *Thamnophis ordinoides* taken in the vicinity of Berkeley in June and July, 1932. The young flukes that were raised experimentally by feeding *Thamnophis ordinoides* with infected *Hyla regilla* tadpoles were mature enough, however, to show all the generic and most of the specific characters. On comparison with young flukes of like size in the intestine of the snakes which also contained the adult flukes they were found to be alike in every detail, so that, while experimental proof is lacking, there is little doubt that the young flukes in both occurrences observed belong to the same species as do the adults.

The adult of *Zeugorchis syntomentera* was described in detail by Sumwalt (1926), so only a general description with supplementary notes needs to be given here.

Twenty-six garter snakes, *Thamnophis ordinoides*, from the vicinity of Berkeley, California, were examined between August, 1931, and July, 1932, and only four contained flukes of this species. The heaviest infection came from a medium-sized snake from a pond in Wildcat Canyon where *Hyla* tadpoles are abundant. This snake had two adult flukes in the mouth, one adult in the oesophagus, and about thirty young flukes, of sizes varying down to metacercarial dimensions, in the intestine. The only other snake with adult flukes harbored two in the mouth, but had no immature forms. This might suggest that the natural habitat for the adult is the mouth, and that possibly the eggs escape from that unusual location.

The average length of five mature flukes is 1 097 mm.; the width, 0.64 mm. The average dimensions of the oral sucker are 0.31 mm. wide by 0.26 mm. long. The acetabulum is 0.40 mm. long and 0.39 mm. wide. The oesophagus is 0.097 mm. long. The eggs average 48μ by 24μ. The entire body is covered with minute spines (not shown in figures) which may be sloughed off during fixation. The caeca do not quite reach the testes, which are slightly oval and bilaterally symmetrically placed. Pharyngeal glands are prominent, as is also a scalloping of the anterior part of the pharynx. There is a large seminal vesicle and prostate in the large cirrus sac. The excretory vesicle is very large and irregular and shows up well in individuals stained with Mayer's hemalum. The vitellaria are somewhat in two groups. One group on each side is lateral to each caecum, and the second is posterior to it.

DISCUSSION

It is interesting to compare the stages in the life-history of *Zeugorchis syntomentera* with those of other genera of flukes in the subfamily Reniferinae. Unfortunately, the experimentally completed life-history is still unknown for any single species of this subfamily; but enough is recorded regarding the different stages and hosts to make a comparison profitable.

McCoy (1928) succeeded in rearing the cercariae of three genera of this subfamily by feeding different species of *Physa* with the eggs of *Pneumatophilus variabilis* (Leidy), *Dasymetra conferta* Nicoll, and *Renifer kansensis* Crow. He found that each of these species required a separate and definite parthenita host, that when they developed in another it was a species of the same genus, and that even then the development was feeble and the time required was longer.

Although the time required for the development of *Z. syntomentera* in the parthenita host has not yet been determined, the host itself is a *Physa,* identified by Dr. Hanna as *Physa gyrina.* It might be added here that the species of *Physa* on the west coast are so poorly known that competent conchologists have suspected the presence of several undescribed species. The failure of the eggs of *Z. syntomentera* to develop in two feeding experiments in which *"Physa gyrina"* was used from a pond where infected snakes were never taken might be explained if the species of *Physa* in this pond is different from that where the infected snakes are found to harbor the infection.

The evidence at hand indicates that species of the subfamily Reniferinae may each require a definite and distinct species of *Physa* which must eat the eggs before the miracidia emerge. It is also known that the cercariae develop in sporocysts. The cercariae of the four genera are strikingly similar in general shape, stylet, stylet glands, and the Y-shaped excretory vesicle with the arms extending at least anterior to the acetabulum.

The metacercaria of *Pneumatophilus variabilis* is not known, and, although McCoy (1928) does not figure this stage for *Dasymetra conferta, Renifer* sp., and *Renifer kansensis,* his descriptions of the method of encystment, structure, and adolescaria host (which is a tadpole for the first two species and a catfish for *R. kansensis*), suggest a parallel to that given here for *Z. syntomentera.*

While the adults have never been raised experimentally, the structural similarities of the young flukes that were grown in the laboratory, connected with several observations made in nature, convince us of the identity of these flukes which were experimentally raised from cercariae and those found in nature. The mature and young flukes agree in the shortness of the caeca, position of the testes, pharynx, position of the female genitalia, and the Y-shaped excretory system. This last feature, although not shown in the figures of stained specimens, was observed in the young living flukes by the writer and also by Sumwalt (1926). The scalloped pharynx, characteristic of the adult, has also been found in young flukes taken from a snake nine days after it had been fed the metacercariae from a *Hyla regilla* tadpole. The young flukes found in the intestine of snakes in nature are indistinguishable from those raised in the laboratory. Over fifty specimens of *Thamnophis ordinoides* and *T. sirtalis* have been examined from different parts of California and no other genus of fluke of this subfamily has yet been taken in this State. All these facts indicate that the young laboratory-raised flukes are the same species as the young and adult flukes taken in the alimentary tracts of *T. ordinoides* collected from their natural haunts.

Baer (1924) excluded *Pneumatophilus* Ohdner from his family Reniferidae and defined the family in such a way that *Zeugorchis* cannot be included, although he did include it in his final list of genera of that family. In the description of the family he states, "genital pore situated laterally in front of the ventral sucker." This does not admit *Zeugorchis,* because here the pore is ventral and median and in the fork of the caeca. In view of McCoy's work (1928) on the life-history of *Pneumatophilus variabilis* (Leidy) and the present work on *Z. syntomentera,* there is little doubt that both belong to the same family and subfamily as do *Dasymetra* and *Renifer*. It seems, therefore, that Baer is unwarranted in excluding in his description of his Reniferidae both *Pneumatophilus* and *Zeugorchis,* and in this paper Reniferinae has been considered a subfamily, as defined by Pratt (1903), of the family Plagiorchiidae.

SUMMARY

The life-history and structure of the stages of *Zeugorchis syntomentera* Sumwalt have been presented.

Physa gyrina Say was found to be the parthenita host. The eggs hatch only after they have been ingested by the mollusc.

The cercariae of *Z. syntomentera* penetrate the skin and become metaceraria e in the tadpoles of *Hyla regilla, Rana aurora,* and even in the western newt, *Triturus torosus*. They attack the adults of *Hyla regilla* but cannot penetrate the skin.

When the infected tadpoles are fed to young garter snakes, *Thamnophis ordinoides,* the young flukes continue to develop in the alimentary tract. The adult fluke lives in the mouth, and its eggs probably leave the host by way of the mouth.

The flukes were not reared to maturity in the experimentally fed snakes, but young flukes secured in this way were indistinguishable from those found in snakes having a natural infection of both mature and young forms. Most of the specific characters appear in the experimentally-raised young flukes. The type of life-history illustrated by *Z. syntomentera* is in close agreement with those known for other species of Reniferinae.

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EXPLANATION OF PLATE 13

PLATE 13

All drawings were made with the aid of a camera lucida.

Zeugorchis syntomentera

Fig. 1. Stained cercaria, ventral view. × 235.

Fig. 2. Young sporocyst. × 163.

Fig. 3. Miracidium, stained intra-vitally. × 850.

Fig. 4. Cercaria drawn from life, ventral view. × 163.

Fig. 5. Egg. × 850.

Fig. 6. Metacercaria. × 63.

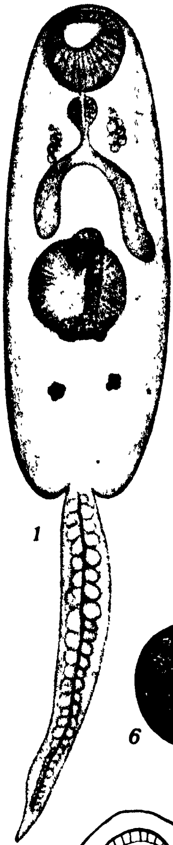
Fig. 7. Acetabulum and part of the genitalia of the cercaria. × 403.

Fig. 8. Stylet of cercaria. × 750.

Fig. 9. Metacercaria removed from cyst, drawn from life, ventral view, showing excretory system. × 118.

Fig. 10. Young fluke, stained, ventral view. × 200.

Fig. 11. Adult fluke, stained, dorsal view. × 53.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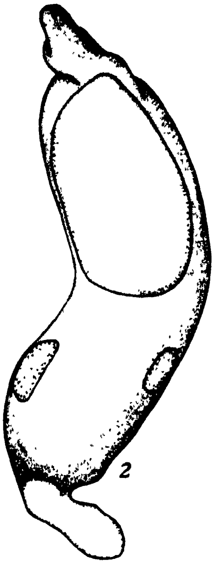
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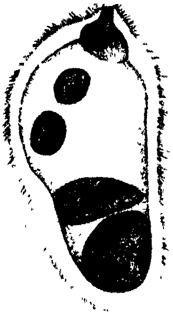
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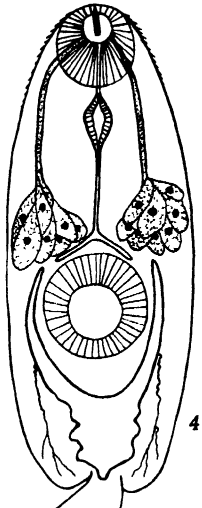
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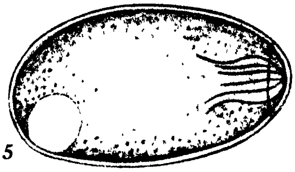
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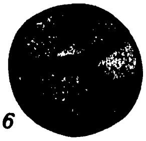
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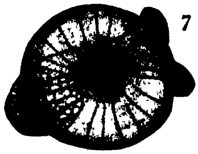
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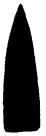
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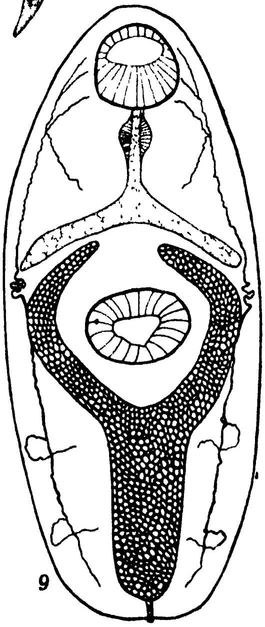
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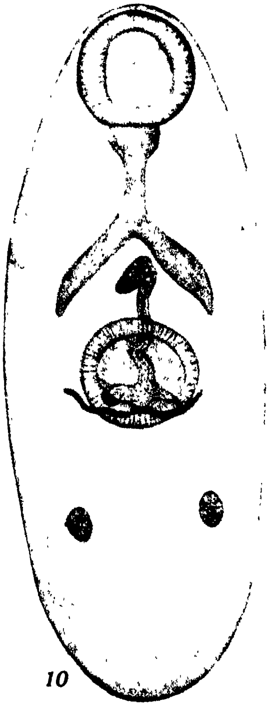
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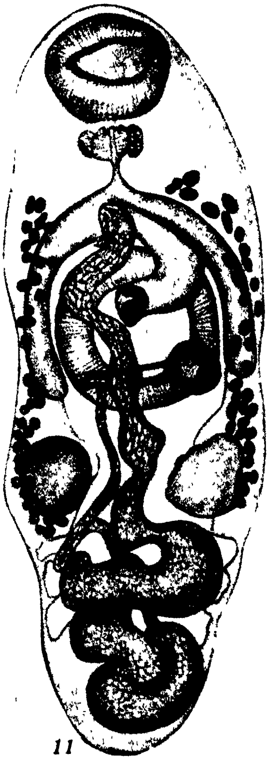
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CORRELATION OF THE DISTRIBUTION OF THE PROTOZOA IN THE INTESTINE OF RATTUS NORVEGICUS WITH THE HYDROGEN ION CONCENTRATION OF THE INTESTINAL CONTENTS AND WALL

BY

C. A. KOFOID, E. McNEIL, AND A. BONESTELL

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BY

CHARLES A. KOFOID, ETHEL McNEIL, AND AILEEN BONESTELL

INTRODUCTION

It was the purpose of a previous paper (Kofoid, McNeil, and Cailleau 1932) to determine the pH of both wall and contents of various parts of the gastro-intestinal tract of the albino rat. As is well known, these rats may be heavily infected with both amoebae and flagellates. Since at least one genus (*Giardia*) is found in wall scrapings rather than in the intestinal contents, a determination of the pH of the wall as well as that of the contents is necessary in any attempt to correlate pH with the presence or absence of Protozoa. The means and standard deviations were computed, and found to be significant in terms of their probable error. The hooded and the brown rat, as well as the albino, were used in the following experiments, but there were no significant differences among them.

When one considers how frequently *Rattus norvegicus* is used for laboratory experiments, it is surprising to find how little detailed information is available concerning the anatomy and physiology of its digestive tract. To make our later statements clearer, we define our conception of the three divisions of the small intestine. The length of the entire small intestine is usually from 101 to 116 cm., although it may be as short as 99 cm., or as long as 127 cm. As defined by Hunt (1924) the duodenum is made up of three parts, a descending, a transverse, and an ascending limb. This definition makes the duodenum not more than 10 cm. in length. The jejunum then ranges from 35 to 45 cm., and the ileum from 55 to 71 cm. in length. There are no obvious lines of demarcation between these regions. Peyer's patches cannot be taken as a criterion for ileum. They are present throughout the length of the small intestine, even in the duodenum, and as many as 20 patches have been found in one individual. Nor can the meaning of the word jejunum (empty intestine) be applied here as strictly as in carnivorous animals, since food is often found in the lower half of the jejunum in the rat. Wall scrapings were taken at intervals of 3 inches throughout the length of the small intestine of six rats.

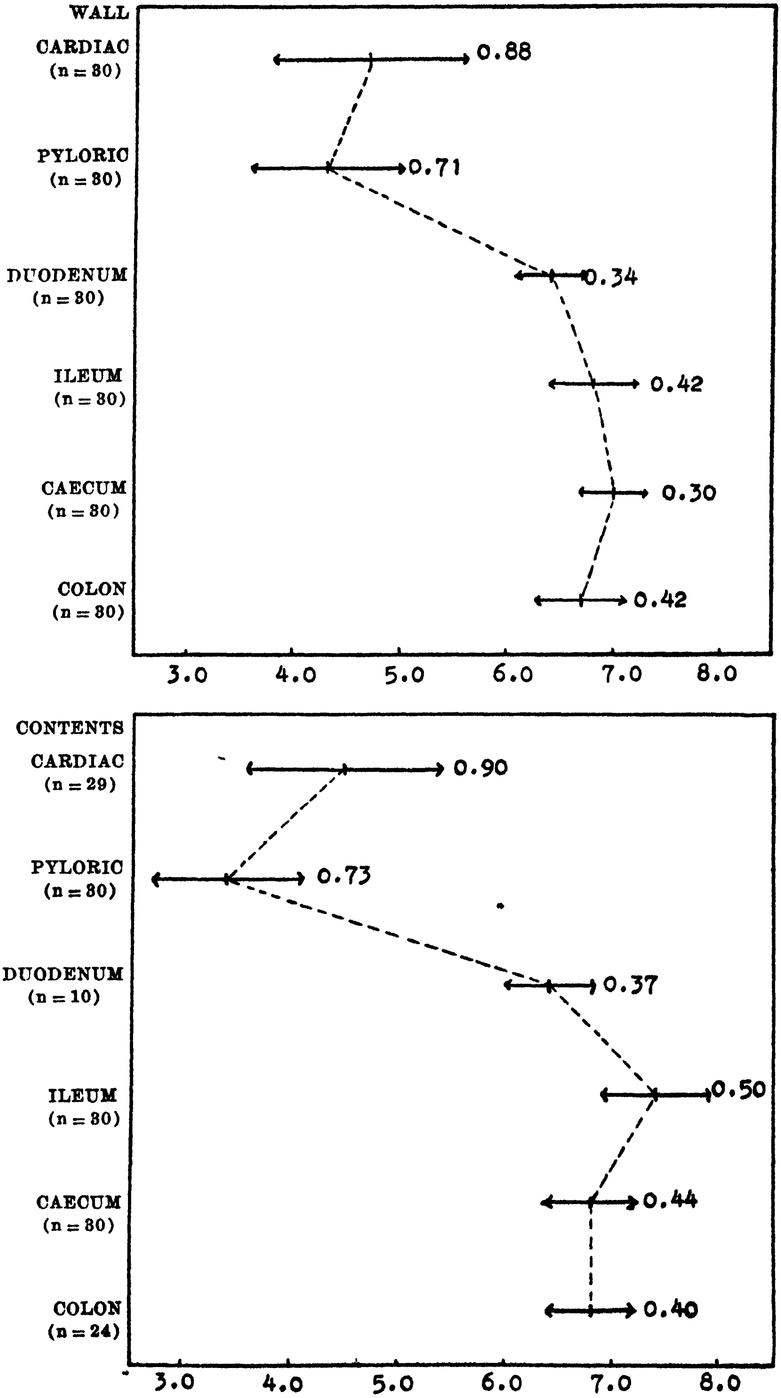

Fig. 1. The hydrogen-ion concentration of the wall and contents of various parts of the gastro-intestinal tract of *Rattus norvegicus*, showing one standard deviation, numerically expressed, above and below the mean.

Beginning with the wall immediately posterior to the pyloric valve, the pH averages were as follows: 5.93, 6.38, 6.45, 6.47, 6.52, 6.47, 6.45, 6.59, 6.59, 6.59, 6.67, 6.75, 6.81, 6.82, 6.79, 6.82. In other words, there is a gradual decrease in acidity posteriorly throughout the small intestine, but no sudden changes corresponding to any definite divisions. The intestinal secretions and food and bacterial contents of the lumen have a definite effect on the pH and thus on the distribution of at least two of the protozoan parasites of the intestine.

In the present paper two series of rats were used. In the first series (nos. 1–30) a general survey of the Protozoa in all parts of the digestive tract was made, and an attempt made to correlate their presence or absence with the hydrogen ion concentration. The second series (nos. A–J) was used for a detailed study of the distribution of the trophozoites of *Giardia.*

The rats were kept on the following routine diet:

	Per cent by weight
Ground wheat	67.5
Alfalfa meal 20% protein	10.5
Fish meal	10.5
Fish oil (Sardeline)	5.25
Casein	5.25
Salt	1.00
pH of the above diet	5.5

METHOD

In general, the same method was used as in the previous work (Kofoid, McNeil, and Cailleau, 1932). The rat was anesthetized with ether and kept alive until all samples were obtained. In this way are eliminated errors due to the effect of abnormal tissue on such Protozoa as *Giardia* and *Hexamitus.* The rat was opened and two samples obtained from each location. One sample was diluted with Locke's solution and examined immediately for Protozoa. At the same time a second worker determined the pH of the other sample. Errors due to time were thus obviated. The quinhydrone reference electrode was used exclusively, since it covers the required pH range and can be manipulated more rapidly than the calomel and hydrogen electrodes.

RESULTS

1. TRICHOMONAS Donné, 1837

The genus *Trichomonas* was found in the caecum of every rat examined (fig. 2), usually in very great numbers, and evenly distributed throughout the caecal material. Contrary to the findings of Hegner (1923) it was also found in the ileum of 40 per cent of the rats, occurring as high as 17 inches above the caecum in one rat. It was also found in the colon in 73 per cent of the rats. The heaviest infections of the ileum with *Trichomonas* were in the 6 inches anterior to the ileocaecal valve. They were present there in great numbers and were very active, but at 8 inches above the caecum they had begun to round up and to decrease in numbers. There seems to be some correlation between this fact and the pH of the intestinal contents. The average pH for the caecal contents is 6.8 (6.15–7.55) (P.E.= ± .08). Above 8 inches the contents of the ileum become increasingly more acid (6.67–5.93), and the movement of *Trichomonas* slower. It is interesting to note that it is always predominantly the *large* form (*T. muris*) that is present in the ileum.

Hegner (1923) states that out of 100 rats examined he found *Trichomonas* in the ileum of only one. In later work he fed *T. muris* to rats and studied its progress down the intestinal tract. He makes the statement that any *Trichomonas* found above the caecum must therefore be from the specimen recently fed to the rats. In view of the fact that we have found *T. muris* in abundance in the ileum of 40 per cent of our rats it appears that his conclusion as to its exclusion from the ileum may not be valid. In all the rats the flagellates were fairly abundant and in 9 rats the ileal infection was definitely heavy. This large number precludes any possibility of their presence being due to freshly ingested faecal material from the cage.

It has been suggested (Hegner, 1924) that the reason for the great abundance of *Trichomonas* in the caecum is the fact that it is carried somewhat passively along the digestive tract, since it has neither the sucking disc of *Giardia* nor the rapidity of motion of *Hexamitus,* which enable them to remain in the upper parts of the intestine. However, both *in vivo* and *in vitro, Trichomonas* can attach itself to the intestinal epithelium by the axostyle and beat violently against the wall. It has been observed to stay attached *in vitro* in tissue culture from 2 to 3 hours. Furthermore, *Hexamitus,* in spite of its motility, is found most abundantly in the ileum.

Therefore the authors conclude that there is a more fundamental physiological reason for the distribution of the intestinal Protozoa than that advanced by Hegner. As yet, we know relatively very little about the secretions at various levels of the rat's alimentary tract. Starling (1920) states that there is an alkaline secretion in the caecum of herbivorous animals, which neutralizes the acid formed by fermentation. Possibly it is this alkaline secretion from the walls of the caecum, which makes it a favorable spot for *Trichomonas* and an unfavorable one for *Giardia.*

It is even more difficult to find information on the physiology of the jejunum. Alvarez in his Mechanics of Digestion says that "the food is whisked rapidly through the upper jejunum and moves more slowly in the lower ileum." Since *Trichomonas* is found throughout the caecal material, it is perhaps less dependent on secretions. Possibly this is why we can culture *Trichomonas,* but are unable to culture *Giardia.*

There is much disagreement concerning the species of *Trichomonas* present in the rat. Ratcliffe (1929) and Wenrich (1924) now recognize three species, *T. muris, T. parva,* and *T. minuta.* The last named species was described by Wenrich as being from 3–10μ in length and possessing three (or four?) chromosomes. *T. muris* measures 8–20μ in length and has six chromosomes. *T. parva* is intermediate in size (5–11μ), and presumably has six chromosomes, although Wenrich in his key to intestinal flagellates of rats does not give the chromosome number. There seems to be considerable confusion in the literature about the identity of *T. parva,* since in 1921 Wenrich discovered that a smaller species of *Trichomonas* had only three (or four?) chromosomes instead of the six of *T. muris.* At the time he thought possibly the species was *T. parva* Alexieff. Therefore Wenyon (1926) in his Protozoology lists *T. parva* as having three chromosomes. Wenrich (1924) lists three species from the rat, this time describing *T. minuta* as having three chromosomes, and inserting after the name *T. parva* "tentative identification." In 1930 Wenrich lists all three species in his key to the intestinal flagellates of rats, but neglects to state the chromosome number for *T. parva.* The discovery of three chromosomes in *T. minuta* has not as yet been confirmed by other workers. He (Wenrich) states that in *T. parva* the undulating membrane is not well developed and that the chromatic basal rod is replaced by a row of chromatic granules.

Ratcliffe (1929) accepts three species and believes that *T. muris* can be separated from *T. parva* by culture methods. According to him only *T. parva* can be cultured, *T. muris* dying out immediately. However, at the present time we have three strains of rat *Trichomonas* in Locke's-egg-serum, in which both sizes are present. Sheep serum (1:8) and rat serum (2 per cent) have been found to sustain growth most satisfactorily. Strain no. 1 is now 6 months old, strain no. 2, 4 months old, and strain no. 3, 4 weeks old. The addition of rice starch results in an increased rate of multiplication. *Trichomonas* first appears in the caecum of rats 3½–4 weeks old, presumably when the bacterial flora is changed following weaning.

2. CHILOMASTIX BETTENCOURTI (Fonseca, 1915)

Of all the rat Protozoa we found *Chilomastix* to be least variable. Motile forms were present in the caecum of 60 per cent and in the colon of 27 per cent of the rats examined (fig. 2). It was never found above the ileocaecal valve.

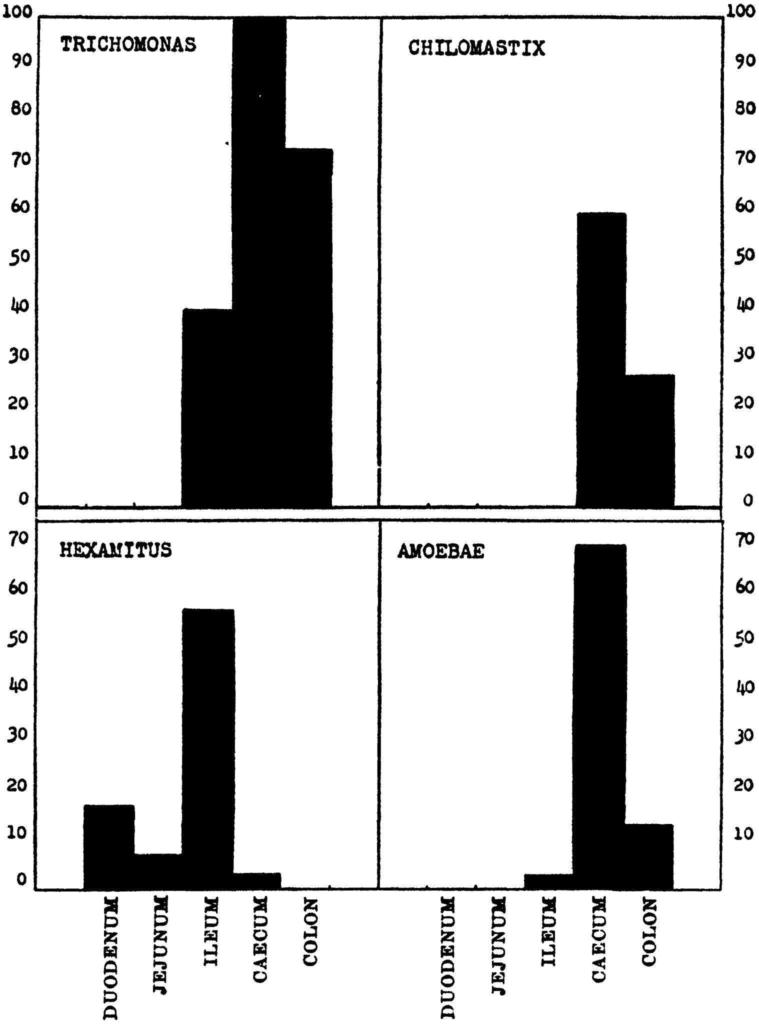

Fig. 2. Percentage frequency distribution of *Trichomonas, Chilomastix, Hexamitus*, and amoebae in the different parts of the intestine of *Rattus norvegicus*.

3. HEXAMITUS MURIS (Grassi 1881)

Hexamitus muris is a small, very slender (7–10 × 3–5μ) flagellate which is mainly interesting because of its extremely rapid movement and because other species of the genus have been reported from the blood stream of amphibians and reptiles. Lavier and Galliard (1925) report a species from the peripheral blood of *Bufo calamita* and cite seven other cases in which *Hexamitus* was found in frogs, toads, and tortoises. So far as is known it has never been reported from mammalian blood.

At times *Hexamitus muris* occurs in such great abundance that the microscopic field is a disconcerting mass of these slender flagellates, whose darting movement is so rapid that it is very difficult to follow individuals. The picture is very different from that in material from the caecum, where the microscopic field is often a wriggling mass of *Trichomonas*. *Hexamitus muris* is found throughout the small intestine and caecum, but is most abundant in the upper ileum, where it is present in 57 per cent of the rats in series 1–30. It was found in the duodenum in 17 per cent, the jejunum in 7 per cent, and the caecum in 3 per cent of the rats of this series (fig. 2). It seems to be the most adaptable of all the Protozoa of the rat, since it is found at a pH range of 5.98–8.27.

4. GIARDIA Kunstler, 1882

Of all the intestinal Protozoa of the rat, *Giardia* is most nearly a tissue parasite. It was found in 60 per cent of all rats examined, always in close proximity to the wall. They frequently attach themselves to the intestinal epithelium by their sucking disc, and except for slight movements of their flagella show no motion whatsoever. Therefore, unless one takes samples of wall scrapings he may easily fail to secure them.

The distribution of *Giardia* is so different from that of the other Protozoa that it deserves special attention. Hegner (1923) states that it is most abundant in the duodenum. But unfortunately he does not state exactly what he means by duodenum, nor do other writers. We accept Hunt's definition that it consists of three parts, a descending, a transverse, and an ascending limb. Therefore it is not more than 4 inches long. We have found that in heavy infections, the focus of the infection is in the jejunum, usually between 9 inches and 15 inches posterior to the stomach. The distribution of *Giardia* in the second series (A–J) is shown in table 1. In rat F the focus of infection is definitely localized in the area between 6 inches and 12 inches. In rat I the same condition is found, the infection is heaviest between 6 inches and 9 inches. In rats A, B and J, where only an extremely light infection was present, the duodenum was negative for *Giardia* as was also the lower ileum. In one exceptional case (H) an unusually heavy infection was present with the result that the entire duodenum and ileum were positive to a one plus degree, with the focus of infection at 36 inches posterior to the stomach. It is interesting to note, however, that in this case the pH of the wall at 36 inches was 6.43, in comparison with the usual 6.82 for that region. This shows definitely that *Giardia* is most abundant at a lower hydrogen-ion concentration than are the other Protozoa. The average pH for the wall of the intestine between 9 and 15 inches ranges from 6.45–6.52.

In no adult rat have we found motile *Giardia* below the ileocaecal valve. This may very probably be due to the alkaline secretion of the wall mentioned by Starling. We have, however, found motile forms in young rats from 20 to 26 days old. One litter was biopsied from the 12th to the 28th day. At 12 days no Protozoa were present; at 21 days and at 22 days there were motile *Giar-*

TABLE 1

DISTRIBUTION OF GIARDIA IN INTESTINAL TRACT

Inches below stomach	Rat *A*	*B*	*F*	*H*	*I*	*J*
1	....	....	++	+	++	....
3	....	....	++	+	+++	....
6	....	....	++++	+	+++	....
9	....	....	++++	+	++++	... .
12	....	....	++++	+	+	....
15	....	....	++	++	+	....
18	Less than 1 per field	....	++	+++	+	1 or 2 *Giardia*
21	....	1 to 2 per field	++	+++	++	. ... .
24	Less than 1 per field	....	++	++++	+	2 or 3 *Giardia*
27	....	....	+	++++	+	. . ..
30	Less than 1 per field	Less than 1 per field	+	++++	1 or 2 *Giardia*	....
33	....	....	1 or 2 *Giardia*	++++	....	....
36	....	....	....	+++++	....	1 *Giardia*
39	....	....	....	+++	....	....
42	....	....	1 or 2 *Giardia*	....	....	....
45	....	....	....	++	1 *Giardia*	....

dia, but no other Protozoa; at 26 days *Giardia* was still present and *Trichomonas* was beginning to appear. After this time *Trichomonas* was always present, but *Giardia* disappeared from the caecum. Another litter gave approximately the same results. Probably the change in diet, after weaning, calls forth the alkaline secretion and results in the disappearance of *Giardia.*

The question of pathogenicity of *Giardia* for the rat has been in dispute. We would agree with Wenrich that the question has not as yet been settled. But we observed that at least some of the rats which showed a heavy infection with *Giardia* also showed intestinal disturbances in the form of liquid faeces and local inflammation.

Potter (1928) has reported two species of *Giardia* from the rat, *G. muris* Grassi and *G. lamblia* Stiles, the former measuring 10.11 × 7.32μ and the latter 17 × 9μ. We have also observed these two sizes in the rats examined by us.

We may conclude, therefore, that *Giardia* is primarily not an infection of the duodenum, but of the upper jejunum. When the infection is heavy, however, it may extend up into the duodenum and down to the lower levels of the ileum.

5. AMOEBAE

Motile amoebae were fairly abundant in the caecum in 21 (70 per cent) of the rats examined (fig. 2). They were also found once in the ileum and four times in the colon immediately adjacent to the caecum. Cysts were, of course, found in the colon in all rats in which motile amoebae were present in the caecum. The amoebae, so far as identifiable, were principally *Councilmania decumani.*

The trophozoites are distributed throughout the caecal material at an average pH of 6.8 (P.E.= ± .08). It is interesting to note (fig. 3) that the curves of the mean pH values for the regions in which all other Protozoa are found, follow each other consistently, while that for amoebae drops conspicuously at the caecum and colon.

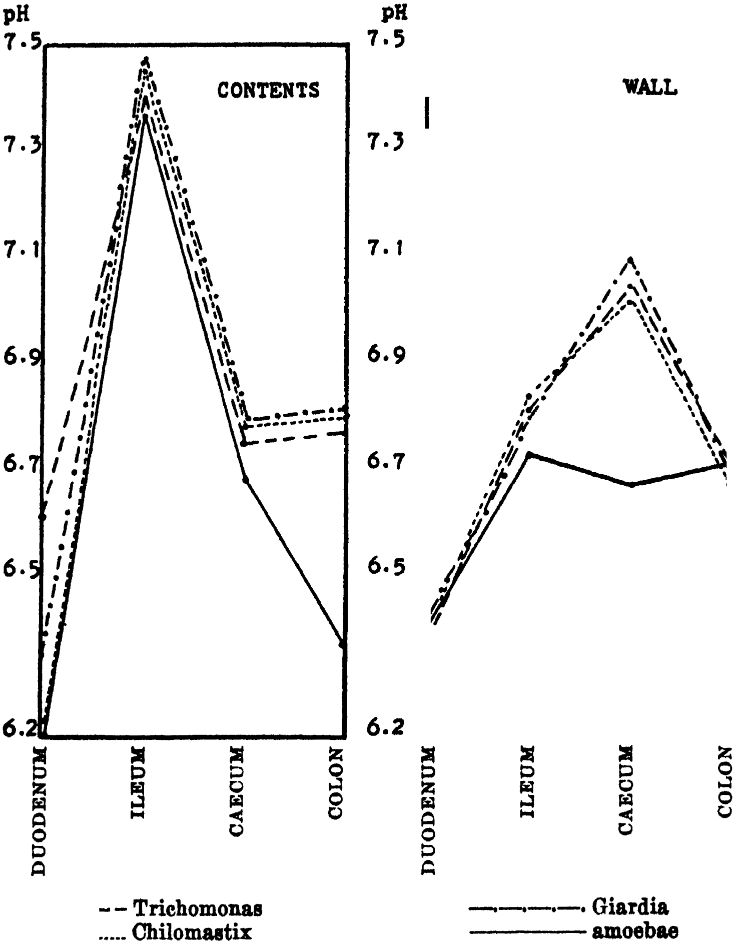

Fig. 3. The solid line is plotted to show the mean pH values of the various parts of the intestinal tract of the twenty-one rats in which amoebae were found. The other curves represent, respectively, the mean pH values of the same parts of the intestine for those rats infected with amoebae, *Trichomonas*, *Chilomastix*, and *Giardia*.

SUMMARY AND CONCLUSIONS

1. *Trichomonas* was found in the caecum of all rats examined. It was also present in the ileum in 40 per cent and in the colon in 80 per cent. There is a correlation between the decrease in numbers of *Trichomonas* in the upper ileum and the decrease in pH value of that region.

2. *Hexamitus muris* was present in the duodenum in 17 per cent, the jejunum in 7 per cent, the ileum in 57 per cent, and the caecum in 3 per cent of the rats examined. They are present at a pH range of 5.98–8.27.

3. *Chilomastix bettencourti* was present in the caeca of 60 per cent of the rats examined, at an average pH of 6.77. It was never found above the ileocaecal valve. It was present in the colon in 8 per cent of the cases.

4. *Giardia* was present in 60 per cent of all rats examined. It is always found in close proximity to the wall. Giardiasis is primarily an infection of the jejunum, occurring in greatest abundance at a pH range of 6.45–6.52.

5. Motile amoebae were present in 70 per cent of the rats examined, at an average pH of 6.7.

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REGULATORY DEVELOPMENT IN TRITURUS TOROSUS (RATHKE)

BY

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INTRODUCTION

The problems of determination and differentiation in Amphibia have been approached largely through analyses of the events in normal development. Perhaps the most rewarding stage for the study of these problems is gastrulation. The periods during and immediately following gastrulation have been characterized by Daniel (1932) as moments *par excellence* for the production of new organs and new systems. Many of the dynamic features in the creation of these new structures have been revealed or clarified by Spemann (1930) and Vogt (1925) and their collaborators. Their observations and experiments on *Triton* especially have been exceedingly fruitful and have contributed greatly to our knowledge of this formative period.

Another avenue of approach to determination and differentiation is offered by the study of regulatory development. The course of development may be upset in some way but the organism will manifest a tendency to right itself and produce a normal individual. Unfortunately, many of the methods used to disturb the norm, such as wounding or subjection to cold, have resulted in bizarre creatures which in many cases present a maze too complicated for analysis. A procedure, however, which will alter the mode in which normal and characteristic structures are created should lend critical information upon the above problems. The supreme attribute of this procedure should be simplicity; for intelligible results and accurate interpretation, a clear insight into the operating forces of the method is essential.

This paper presents a new experiment concerning regulatory development in *Triturus torosus*, the Pacific coast newt, and indicates some of the general results obtained. I undertook this study at the suggestion of Professor J. Frank Daniel, to whom I express sincere gratitude for his gracious and valuable advice.

METHOD

Because of the large amount of yolk the gastrulae of *Triturus torosus* are relatively fragile and highly susceptible to disturbing influences. Variation in osmotic pressure, mechanical injury, and subjection to low temperatures produce abberant forms which seldom manifest complete regulation.

If, however, one inject gelatin into the segmentation cavity of a blastula, the normal mechanics of gastrulation can be altered without initiating general disorder. A colloid, the solidifying point of which is approximately 19° C is obtained by dissolving about 5 grams of Difco gelatin in 200 cubic centimeters of hot water. Sufficient neutral red (2 per cent solution) is added to impart a rich color; without the red tint the flow from the injection canula is imperceptible. The gelatin is then filtered and sterilized in the autoclave. All instruments, glassware, syringes, and needles are likewise sterilized. Holtfreter's solution (Holtfreter, 1931) and Schotté's silk rings (Schotté, 1930) upon which to maintain embryos are used exclusively. Blastulae to be injected are thoroughly washed by passing through several baths of Holtfreter's solution.

The injection apparatus (fig. A) consists of a two-cubic centimeter Luer syringe (*sy.*^{1}) and a three-inch steel needle (*n.*) tipped with a fine glass canula (*c.*) of approximately 0.2 millimeter in diameter. Large glass tubing provides thin-walled canulae which may be firmly sealed on the steel needle with DeKhotinsky's cement. Some device is necessary to obtain firm and precise depression and elevation of the canula. A modified microdissection apparatus which was at my disposal fulfilled this requirement. The syringe assumes a horizontal position when firmly clamped on the arm (*a.*) of the dissection appartus; hence, the steel needle is bent midway to provide a vertical direction of the canula. The screw (*sc.*^{1}) moves the canula in a horizontal plane, while a second screw (*sc.*^{2}) accomplishes a vertical movement. A finer and more facile control than the ordinary plunger is imperative. A glass tube (*t.*) is therefore lead from the plunger end of the syringe (*sy.*^{1}) through a rubber stopper (*s.*) and is inserted into a rubber tube (*r.*) of four millimeters in diameter. The tube is connected to a one-half cubic centimeter Luer syringe (*sy.*^{2}) which may be operated conveniently at the side of the microscope. The entire system from second syringe (*sy.*^{2}) to canula is filled with gelatin; pressure is thus applied through a continuous liquid medium. This method obviates the unevenness and resiliency of air pressure.

A small petri dish, the floor of which has been covered with a layer of beeswax, is filled with Holtfreter's solution and placed on the stage of a dissecting microscope. A blastula to be injected is oriented in a depression in the wax and a small aperture in the region of the animal pole is created by thrusting a fine needle knife through both chorion and roof of the blastocoel. A gentle flow of gelatin is initiated by slight pressure of the plunger of the second syringe (*sy.*^{2}) and the canula is then inserted through the aperture into the blastocoel. An approximately uniform stream of gelatin is maintained throughout the experiment thereby permitting variation of amount injected by mere alteration of the time element. When the blastocoel has been filled, the canula is withdrawn and the specimen immediately transferred to a container of cold water, the temperature of which ranges from 12° to 15° C. The gelatin within quickly solidifies but will not melt again unless the temperature is raised to the proximity of 23° C. This last feature insures maintaining injected individuals at 20° C. Controls for each series are likewise subjected to puncture and cold water.

RESULTS

The puncture produced in the chorion for the purpose of injection is apparently closed, thereby preserving the protective rôle of this investing membrane. The chorion not only serves as a possible defense against bacterial infection but also facilitates preserving the normal shape and even contour of the developing embryo. The wound sustained by the roof of the blastocoel, however, does not always heal. If the blastocoel is too heavily injected the pressure of the gelatin within may gradually enlarge the aperture. Thus, in certain cases a window is produced which is in itself, a novel creation, for it permits a view of the interior of the blastula. Observed through the transparent gelatin the size and disposition of the yolk cells can be clearly determined. This internal view of the blastula also enables one to judge the relative thickness of various regions in the animal hemisphere and to perceive the activity of outer pigmented, yolk, and especially mesodermal cells. Specimens in which too much gelatin has been injected eventually produce monstrosities which may be beyond interpretation.

Most blastulae early repair the injury sustained from injection and maintain a normal shape and superficial appearance. A slight circumferential depression, however, marks the boundary between the gelatin and the roof of the yolk (see pl. 14, fig. 3). Thus, a meridional section of the blastula would disclose a semicircular cap of ectodermal cells over the gelatin, below which is the solid mass of yolk cells. The first indication of gastrulation is the appearance of the blastoporal groove which forms in the region below the white shield. The discernible features of gastrulation in this experiment vary considerably from those of the norm. Invagination and typical involution are apparently prevented by the obstruction of the gelatin. Moreover, rotation does not take place.

The advent of the medullary plate is first indicated by concentrating pigment. One significant difference exists between neurulae of the controls and of experimentals. The normal medullary plate (*mp.*, pl. 14, fig. 1) forms across the animal hemisphere. The experimental, however, is in direct antithesis to this (pl. 14, fig. 3). In it the medullary plate appears across the vegetative hemisphere; hence it is necessary to invert the embryo completely in order to observe the forming nervous system (*nt.*). Neural folds subsequently form and fuse first in the otic region and from thence caudad and cephalad. By this time cranial flexure is the marked feature of the control.

Schechtman (1930) has shown for *Triturus torosus* that stains placed on the animal pole of the zygote or on the intersection of the first two cleavage planes will mark the general location of the transverse fold of the neurula. Stained controls of the present experiment confirm these results. Experimentals are likewise stained with neutral red at the point of injection, which is approximately on the upper pole of a meridional axis. This marked area shows no migration and remains as the center of the ectodermal cap some 180 degrees from the center of the medullary plate. Thus, the general region of the animal pole, which in urodeles normally approximates the transverse fold of the medullary plate or adjacent epidermis, lies far distant from the nervous system that is actually produced in the experimental animal.

A gross dissection of the medullary plate (*mp.*, pl. 14, fig. 4) of an experimental can be performed easily by the use of needle knives. After the chorion has been removed a longitudinal incision is made along the medullary groove. Each half of the plate (*mp.*) is then gently turned to the side thereby revealing an underlying layer of chordamesoderm (*cm.*) in the form of a tongue. This tongue-shaped layer is composed of cells which are small, spherical, and slightly gray in color and can be distinguished clearly from the yolk cells (*yk.*) visible on both sides. The foremost extension of the chordamesoderm is directly beneath the brain region and its anterior boundary underlies the transverse fold of the medullary plate. In some specimens, particularly in the posterior region, the notochord can be recognized as a column of cells occupying a median longitudinal position.

When blastulae showing the first indication of the blastoporal groove are injected as above, the area of the organizer of Spemann can be stained with certainty. Upon later dissection of specimens so stained the colored region is seen to extend along the median line of the chordamesoderm. This layer of chordamesoderm can be turned to the side as in the case of the medullary plate and immediately beneath it is the dense mass of yolk cells. The gelatin in the form of a hemisphere, the base of which rests upon the yolk and the surface of which is capped with a thin layer of ectodermal cells, is readily disclosed upon folding back the yolk cells. In many cases the cells of the ectodermal cap are tightly adhered to the gelatin and require a needle knife to strip them off.

Transverse sections (pl. 14, fig. 5) of embryos which have been maintained for a week after injection show the degree of internal differentiation attained. The epidermal cap (*an.*) covering the gelatine (*g.*) is continuous over the vegetative hemisphere. While the epidermis above the gelatin may become several cells in thickness, it forms only skin. In a midventral position and inside the epidermal layer is located the neural tube (*nt.*). Central to the neural tube is the notochord (*ch.*) and on both sides lie the somites (*so.*) which are continuous with the lateral plates (*lp.*). The formation of a notochordal sheath is an evidence of further differentiation. The lateral plate has already differentiated into somatic and splanchnic layers between which is an incipient body cavity. These mesodermal layers terminate at the floor of the gelatin, the covering of the animal hemisphere being solely ectodermal. Central to the notochord and splanchnic mesoderm is the compact body of yolk cells (*yk.*). Above the notochord and entirely within the yolk a small cavity (*dg.*) occurs which can be traced through many sections. This appears to be the beginning of the lining of the digestive tract, the creation of which is wholly different from that of a normal individual.

If one select heavily yolk-laden blastulae and inject a large amount of gelatin, an added mechanical difficulty to gastrulation is encountered. The gelatin prevents invagination and ingrowth of the dorsal lip under the animal hemisphere, while an extremely large yolk plug precludes direct epiboly. The appearance of the neural folds (*nf.* pl. 14, fig. 2) under these conditions is much like the "Ring embryos" obtained by Goerttler (1926). Virtually encircling the base of the epidermal cap (*an.*) are the two neural folds (*nf.*) which converge at the posterior region. Below the neural folds is the hemispherical yolk plug (*yk.*). Upon gross dissection it was found that immediately beneath each neural fold there lies the unmistakable layer of mesoderm which extends forward around the base of the gelatin, and on to the tip of each neural fold.

Further results and interpretations will be presented in a later paper.

SUMMARY

1. A method for injecting gelatin into the segmentation cavity of a blastula of *Triturus torosus* is here described.

2. This method offers a new approach to the problems of regulatory development, determination, and differentiation.

3. Invagination, typical involution, and rotation are prevented by the gelatin within the blastocoel.

4. A medullary plate forms on the vegetative area; inversion of the embryo is necessary to observe the forming nervous system.

5. The epidermal cap covering the hemisphere of gelatin forms only skin.

6. Gross dissection of the neurula stage of an experimental shows a layer of chordamesoderm in the form of a tongue between the medullary plate and yolk cells.

7. Transverse sections of embryos fixed a week after injection show the degree of internal differentiation which has taken place. Neural tube, notochord, somites, and the beginning formation of the lining of a digestive tract within the yolk are clearly shown.

8. If a heavily yolk-laden blastula is injected with an excessive amount of gelatin a large yolk plug remains which prevents direct epiboly. In this case the neural folds appear about the base of the epidermal cap and converge at the posterior region. This condition resembles the "Ring embryos" obtained by Goerttler (1926).

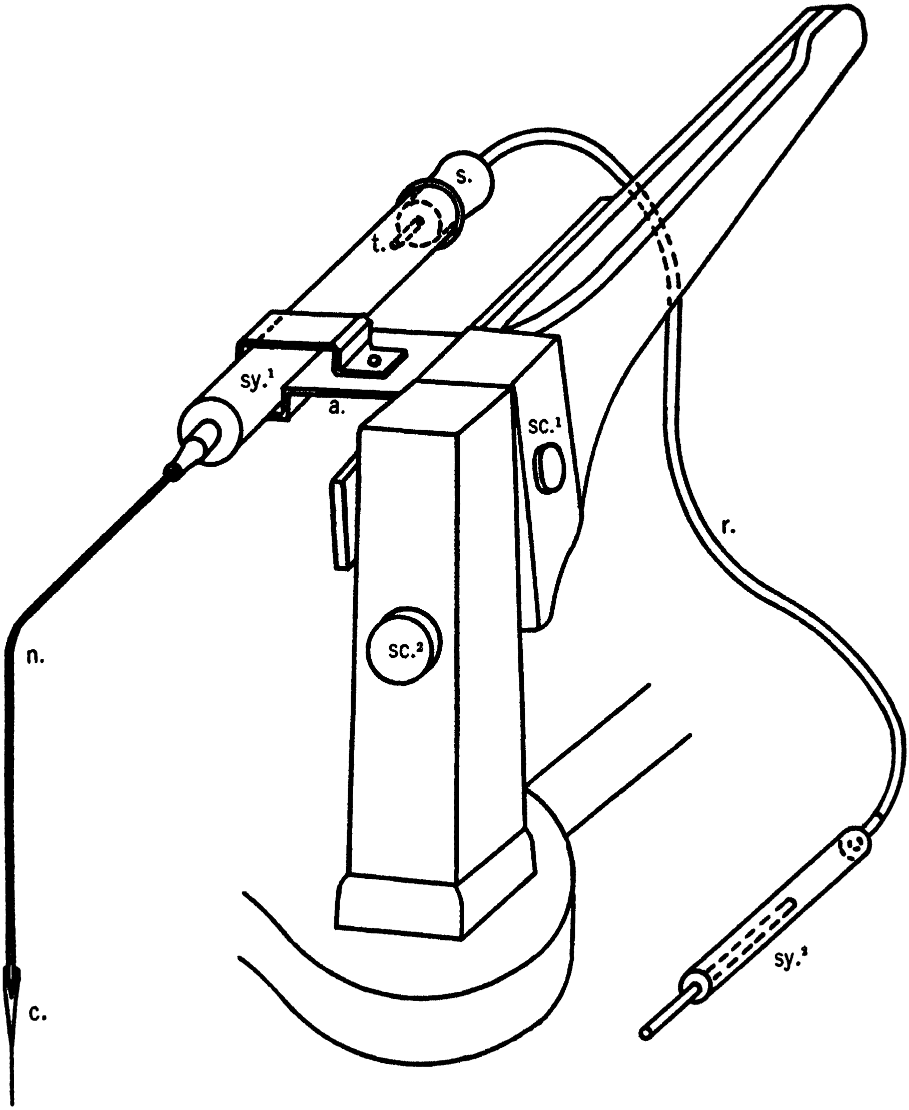

Fig. A. Injection Apparatus. *a.*, arm; *c.*, glass canula; *n.*, steel needle; *r.*, rubber tube; *s.*, rubber stopper; *sc.*^{1}, screw for horizontal adjustment; *sc.*^{2}, screw for vertical adjustment; *sy.*^{1}, two *cc.* Luer syringe; *sy.*^{2}, one-half *cc.* Luer syringe; *t.*, glass tube.

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EXPLANATION OF PLATE

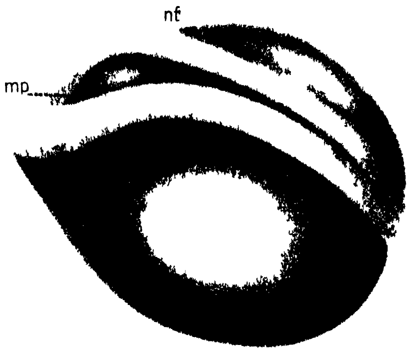
Fig. 1. Normal neurula of *Triturus torosus*, view of left side. *mp.*, medullary plate; *nf.*, neural fold.

Fig. 2. Neurula of an experimental *Triturus torosus*, showing the neural folds converging in the posterior region. *an.*, epidermal cap; *nf.*, neural fold; *vg.*, yolk plug.

Fig. 3. Late neurula of an experimental *Triturus torosus*, rotated to show the vegetative hemisphere. *an.*, epidermal cap; *nt.*, neural tube; *vg.*, vegetative hemisphere.

Fig. 4. Gross dissection of a neurula of an experimental *Triturus torosus*, with the right and left halves of the medullary plate turned to the sides so as to show the chordamesodermal organizer of Spemann. *cm.*, chordamesoderm; *mp.*, medullary plate; *yk.*, yolk cells.

Fig. 5. Transverse section of an experimental embryo of *Triturus torosus*. *an.*, epidermal cap; *ch.*, notochord; *dg.*, digestive tract; *g*, gelatin; *lp.*, lateral plate; *nt.*, neural tube; *so.*, somite; *yk.*, yolk cells.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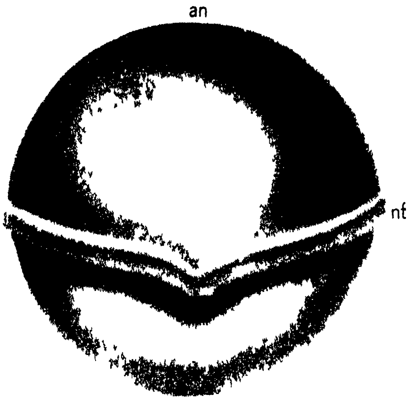
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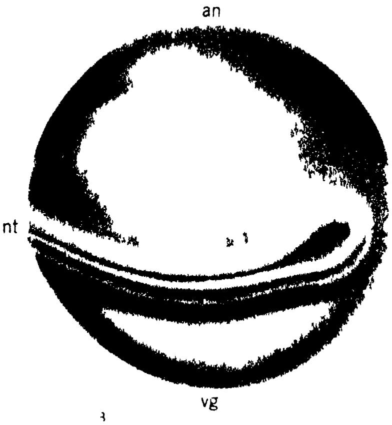
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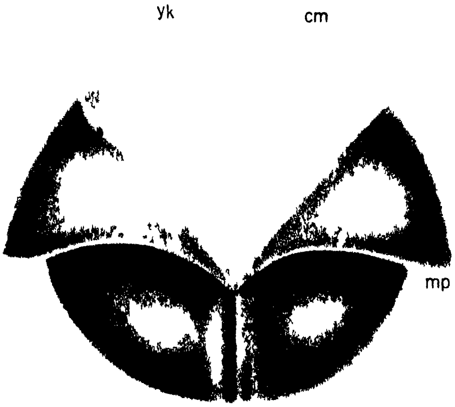
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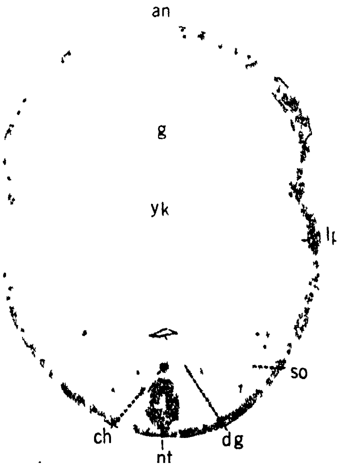
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A ROTARY DISC FOR THE OBSERVATION OF OBJECTS IN PROFILE

BY

J. FRANK DANIEL AND A. B. BURCH

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INTRODUCTION

In the course of a recent experimental study on the development of amphibian eggs it became apparent that a correct interpretation of the progress made in development demanded, first, that the egg be observed from the side and, secondly, that some method be perfected by which the egg could be rotated around its polar axis so that any desired meridian might be studied at any given time.

In an early attempt at observation in profile, a modification of the method which Dellinger (1906) applied to locomotion in Amoeba was tried, but with limited success. Next a small square cell was constructed of cover slips, and this mounted so that it could be rotated horizontally. A prism was used, with the result that a fairly good view of the side was available. Finally, we have perfected a method by which a direct lateral view may be obtained and rotation successfully accomplished.

APPARATUS

The apparatus (figs. A–C) consists of a cylindrical reservoir (*r.*) within which a rotating disc (*d.*) is arranged before the vertical face of a small 90° prism (*p.*), silvered on the oblique face. Thus, when a microscope is focused on the horizontal face of the prism (*p.*), an immersed object (*o.*) mounted on the disc may be examined in profile.

The reservoir, 3.5 centimeters in height and with a capacity of 16 cubic centimeters, is made of glass tubing with an inside diameter of 2.5 centimeters. One end of the tube is closed and flattened to form the base of the reservoir. Into this base the sleeve (*s.*) of a No. 5 standard taper ground glass joint is fused by means of a ring seal, the greater end of the sleeve projecting into the reservoir 2.5 centimeters.

The rotary disc (*d.*) is fused to the top of a ground glass plunger (*pl.*) and a small bead is fused to the center of this disc to serve as a tee on which the observed object rests, thus elevating it above the disc and in the direct line of the prism.

With a thin film of vaseline applied to the bearing surfaces, and the plunger seated in the sleeve, the joint is watertight and the disc may be readily rotated.

The prism (*p.*) with vertical and horizontal faces 1.25 centimeters square is sealed into a square niche ground into the wall of the reservoir (*r.*) so that the horizontal face is flush with the top. The vertical face forms part of the wall itself, thus eliminating the distortion of the image that would result if the light passed through curved or unpolished glass.

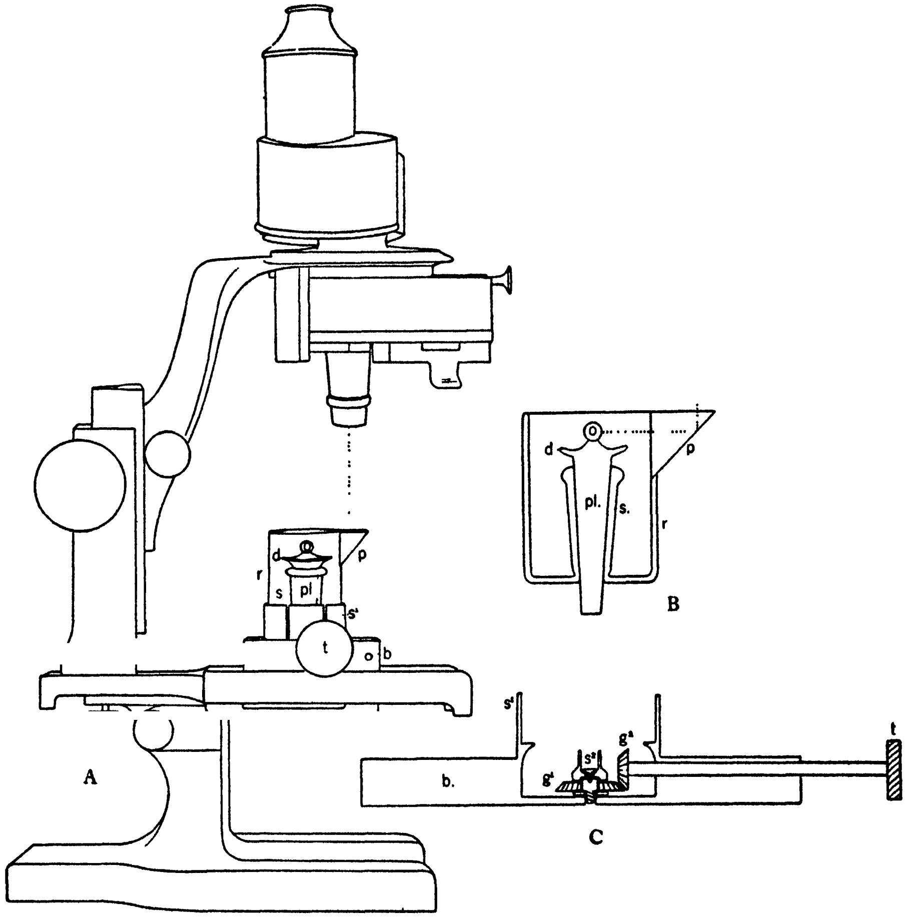

Fig. A. Apparatus in place on microscope. Fig. B. Detailed diagram of valve. Fig. C. Detailed diagram of gear.

b., base; *c.*, position of clamp; *d.*, rotary disc; $g.^{1-2}$, horizontal and vertical bevel gear, respectively; *o.*, object; *p.*, prism; *pl.*, plunger; *r.*, reservoir; *s.*, sleeve; $s.^{1-2}$, sockets for holding reservoir and axial rod of plunger, respectively; *t.*, thumb screw.

A brass mounting or base (*b.*), which in figure A fits into the stage, is provided further with places for adjustable clamps (*c.*) so that attachment may be made to any type of stage. The reservoir (*r.*) is held firmly in place in this base by a large socket ($s.^1$), while the axial rod of the plunger is clamped into a smaller socket ($s.^2$) mounted on a horizontal bevel gear ($g.^1$, fig. C). The horizontal gear is in mesh with a vertical bevel gear ($g.^2$) which is turned by the rod and thumb screw (*t.*)

With an instrument of this type at hand, direct and prolonged observation from the side can be accurately made. Horizontal rotation is readily effected and the movement of the object studied is so smooth that it is not disturbed in any way.

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THE PULSATORY CYCLE OF THE CONTRACTILE VACUOLES IN THE OPHRYOSCOLECIDAE, CILIATES FROM THE STOMACH OF CATTLE

BY

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THE PULSATORY CYCLE OF THE CONTRACTILE VACUOLES IN THE OPHRYOSCOLECIDAE, CILIATES FROM THE STOMACH OF CATTLE

BY

RONALD F. MACLENNAN

INTRODUCTION

The contractile vacuoles of the Protozoa are both obvious and active organelles and have long been the objects of considerable study. In spite of this, the contractile vacuoles of the Ophryoscolecidae have attracted far less attention than any other of the organelles of this large group of ciliates. The vacuoles of the Ophryoscolecidae are large, have a relatively slow pulsatory cycle, are fixed in location, and are covered with a relatively smooth and transparent pellicle. They thus afford highly favorable material for observations on both living and fixed material and also give an opportunity to compare conditions in a number of highly diversified but closely related genera.

The purpose of this paper is to give an account of the pulsatory cycle of the contractile vacuoles in the Ophryoscolecidae with particular emphasis on the following points which are of interest in connection with the problem of contractile vacuoles in general: (1) formation, growth, and discharge of the vacuoles; (2) the sol-gel phenomena exhibited by the cytoplasm during the pulsatory cycle; (3) the excretory pore and the mechanism of its action; (4) the relationship between the vacuole and the various components of the cytoplasm.

I wish to express my appreciation of the very helpful advice and criticism given to me by Professor C. A. Kofoid and Professor Harold Kirby, Jr., during the course of this investigation.

MATERIAL AND METHODS

The Ophryoscolecidae, together with numerous holotrich ciliates, are found in the first, second, and third stomachs of ruminants. They are found in greatest abundance and in best condition in the second stomach (reticulum). The ciliates used in this investigation include one or more species of each of the following genera: *Epidinium, Eudiplodinium, Metadinium, Ophryoscolex, Ostracodinium,* and *Polyplastron.* All genera except *Ophryoscolex* were found in both cattle and sheep; *Ophryoscolex* was found only in sheep.

The material from the second stomach was obtained from cattle and sheep slaughtered in Oakland, California. The contents were removed on the killing floor and brought to the laboratory in tightly corked Dewar flasks. The ciliates remain alive twenty-four hours or longer in material kept in this manner. Preparations sealed with vaseline were studied under a microscope fitted with a warm stage. The activity of the ciliates was controlled by adjusting the temperature of the warm stage.

Comparison of fresh material and material from ten to twelve hours old showed that the ciliates kept in a Dewar flask for more than twelve hours exhibit the beginnings of degeneration of the cytoplasm, the most obvious mark being a general, irregular vacuolization of the ectoplasm. In order to eliminate any artifacts from this source, no material was studied later than six hours after taking it from the host, and no slides of live ciliates were studied later than one hour after they were prepared. In addition, only those ciliates which showed normal action of the membranelle zones were used.

Standard staining methods were followed as closely as possible in order to give results which might be compared with those of the majority of other workers.

Janus green B, Bismarck brown, neutral red, and neutral violet were used to demonstrate mitochrondria and the vacuome in live material by the film method described by Hall (1929). These stains used in one-tenth to one-half per cent solution in the stomach contents are specific for the cytoplasmic granules and are not toxic to the ciliates.

In order to test the reaction to osmic vapor of the granules staining with neutral red ("vacuome" according to Hall, 1931), coverslip preparations of live ciliates were inverted over a depression slide containing 2 per cent osmium tetroxide solution and sealed in place with vaseline. Camera lucida sketches of the arrangement of granules in a restricted region of cytoplasm were made before the neutral red faded out. At frequent intervals over a period of seven days the preparations were compared with the original sketches. The granules stained with neutral red were never selectively impregnated by the osmic treatment, but were darkened along with the general cytoplasm. Occasionally granules which had not been stained with neutral red showed a slight darkening after two or three days. We conclude that these granules which may be stained with neutral red are not osmiophilic, and are not identical with the osmiophilic granules.

Both osmic and silver impregnations were made according to the methods recommended by Bowen (1928*a*) for the demonstration of Golgi material. The silver impregnations were made at room temperature (21° C.), while the osmic impregnations were made both at room temperature and at 32° C. The cold method is far more satisfactory than the warm method. Successful impregnations showed blackened granules in 95 per cent of the ciliates. Some of the ciliates impregnated in osmium tetroxide were stained by Altmann's aniline-fuchsin-picric acid method in order to demonstrate mitochondria along with the osmiophilic granules.

The different species were segregated by means of a fine pipette, imbedded in paraffin stained with Sudan III, and sectioned at two, five, or seven microns.

Small granules in the ectoplasm of all the species investigated and the small prisms of the skeletal plates were found to be stained with Sudan III. Granules similar in size and distribution were stained brown by chlor-zinc-iodide, which is used as a test for glycogen. Several sections stained with Sudan III were drawn with the aid of a camera lucida and then restained with chlor-zinc-iodide. Comparison with the camera lucida sketches showed that the red granules and prisms are stained brown. It seems, therefore, that the above method of using Sudan III reveals glycogen granules.

GENERAL DESCRIPTION OF THE CYTOPLASM

The boundary layer, a thin, hyaline membrane, divides the cytoplasm of the Ophryoscolecidae into ectoplasm and endoplasm. This layer, which is 0.2μ or less in thickness, appears homogeneous in both living and fixed material. A fibrillar structure in the membrane has been described by Eberlein (1895) and more recently by Rees (1931). Sharp (1914) pointed out that the presence of fibrils pressed close to the boundary layer probably led to this interpretation. This criticism is particularly applicable to the genus *Metadinium* which Rees investigated (his *Diplodinium medium*). All our observations bear out the homogeneity of the boundary layer. The cytoplasm is composed of a clear matrix in which are imbedded innumerable spherical granules, from 0.2 to 0.5μ in diameter.

The only movement present in the ectoplasm is Brownian movement of the granules and weak localized currents in connection with the contraction of the vacuoles. In the endoplasm, in addition to pronounced Brownian movement of the granules there is cyclosis. Both parts of the cytoplasm are in the sol state, with the endoplasm much less viscous than the ectoplasm. Localized areas of gelation, which occur at times in the ectoplasm near the contractile vacuoles, will be described in detail in the parts dealing with the vacuolar cycles. No gelation was observed in the endoplasm of normal individuals.

Cytoplasmic granules may be either in direct contact with the matrix, or separated from it by a watery fluid. It was asserted by Taylor (1923) that the granules of *Euplotes patella* lie in a clear, watery vacuole surrounded by an envelope of relatively viscous cytoplasm. In some specimens, he could see this minute vacuole, in others he inferred it from the evident restriction of the Brownian movement of the granules. Mast (1926) reported a similar condition in the gelated portions of *Amoeba proteus.* He based his conclusions on observations of the narrow amplitude of the Brownian movement and on the retention by the granules of a fixed position in relation to each other. The Brownian movement of granules in the ciliates from cattle was studied carefully on this point. No watery vacuoles could be discerned around the granules, and they were continually changing position and tumbling around one another. In the gelated regions, no Brownian movement could be observed. Therefore the granules lie in direct contact with the clear, homogeneous matrix of the cytoplasm and not in small, watery vacuoles.

IDENTIFICATION OF CYTOPLASMIC GRANULES

The granules of the ectoplasm were studied carefully in order to identify those showing relationship to the contractile vacuoles. The only criteria for distinguishing different types of granules in the Ophryoscolecidae are the staining reactions, since the granules are usually indistinguishable in form or size.

Mitochondria.—Mitochondria were identified in living material by staining with Janus green B, and in fixed material by the Champy-Kull method or by staining with acid fuchsin and picric acid after osmic impregnation. The mitochondria are exceedingly numerous small granules uniformly distributed through the ectoplasm. They usually appear to be the smallest of the ectoplasmic granules, their order of magnitude being about 0.2μ.

Glycogen.—Glycogen granules occur abundantly in well fed Ophryoscolecidae. Iodine reagents (sulphuric acid followed by iodine; chlor-zinc-iodide; Lugol's solution) are useful for demonstrating glycogen granules temporarily, but are unsuitable for fine cytological preparations. Sudan III, used in the manner described above, does not interfere with the study of any cytological details and is therefore most useful in attempts to correlate the distribution of glycogen granules with that of other cytoplasmic inclusions.

The scattered glycogen granules are usually in the form of small spheres about 0.5μ in diameter. In chlor-zinc-iodide preparations they appear as homogeneous dark brown bodies with a rather irregular surface, but in sections stained with Sudan III, each granule appears as a smooth pink sphere with a brilliant red granule in the center. Occasionally the glycogen granules of *Ophryoscolex* appear as stout rods up to 1μ in length.

Glycogen is particularly abundant in the skeletal plates (fig. A, *1*), in the postero-ventral region of the body, and in the operculum. Since vacuome, chondriome, or Golgi material is often described in close connection with such reserves of food, a similar relationship was looked for in these ciliates. The glycogen granules, however, showed no close spatial relationship to the other cell inclusions (pl. 15, fig. 14).

Vacuome.—Neutral red stains specifically small spherical inclusions in many types of animal cells. The sum total of these inclusions has recently come to be known as the vacuome (Parat, 1928). Neutral red used on living Ophryoscolecidae consistently stains numerous granules ranging in size from 0.2 to 0.5μ. They are particularly numerous in the ectoplasm which forms the operculum, in that of the postero-ventral region, and in a narrow region just posterior to the membranelle zones (fig. A, *3*). These regions appear as solid red bands under low magnification, but under high magnification it is clearly

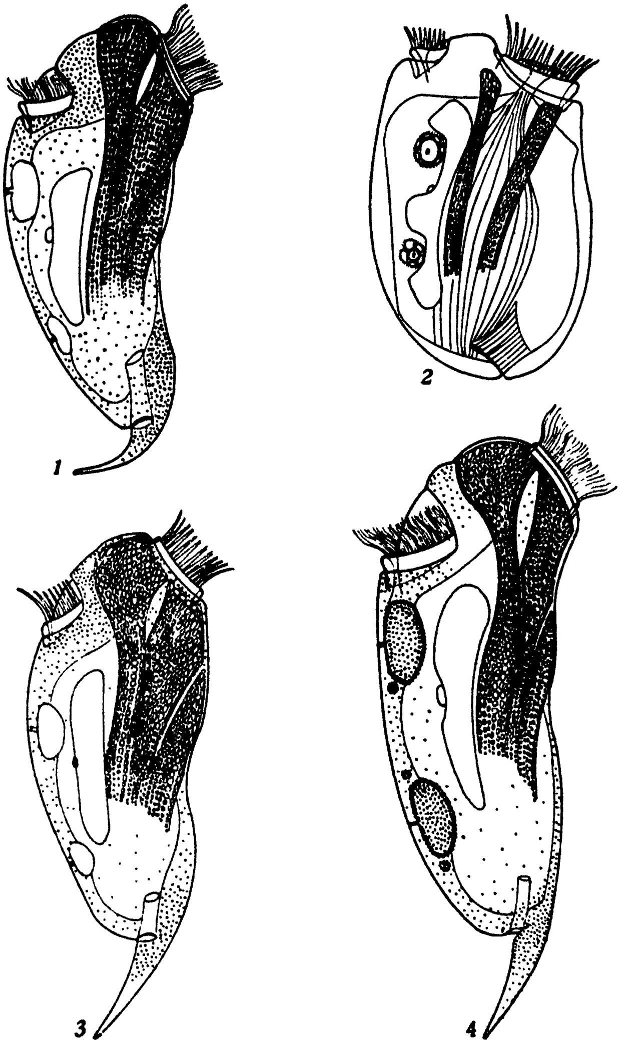

Fig. A. ***1, 3, 4, Epidinium caudatum,*** **× 500,** ***1,*** **stained with chlor-zinc-iodide to show distribution of glycogen;** ***3,*** **stained vitally with neutral red to show distribution of vacuome;** ***4,*** **impregnated with osmium tetroxide to show distribution of osmiophilic granules; semidiagrammatic.**

2, Metadinium medium, **× 250, unstained live specimen, semidiagrammatic.**

seen that this appearance is caused by the staining of many of the granules, some of the granules and the cytoplasmic matrix remaining colorless. A small number of granules which stain with neutral red are scattered uniformly through the rest of the ectoplasm. A few of them usually adhere to the outer surface of the vacuolar membranes, but there is very definitely no concentration of the granules near these structures. The granules described above correspond in position, size, and reaction to neutral red to the vacuome described by Joyet-Lavergne (1926) and Hall (1931) in various Protozoa.

Osmiophilic granules.—Osmic and silver impregnation of the Ophryoscolecidae reveals a large number of granules specifically blackened. These granules are all less than 0.5μ in diameter, the largest ones occurring in *Eudiplodinium,* the smallest in *Polyplastron.* The granules are distributed generally throughout the ectoplasm (except perhaps in *Eudiplodinium*) and at certain times are very heavily concentrated around the contractile vacuoles (fig. A, *4*), sometimes giving the appearance of a heavy, thick, osmiophilic wall around the vacuole.

In the Ophryoscolecidae, the granules stained with neutral red are not blackened by osmic treatment, as pointed out in the discussion on technique; also the neutral red granules (fig. A, *3*) are never concentrated around the vacuoles as are the osmiophilic granules (fig. A, *4*). These observations show unmistakably that, in the Ophryoscolecidae, the neutral red granules and the granules demonstrated by osmic or silver techniques for Golgi materials are not identical; therefore the findings reported by Joyet-Lavergne (1926) and by Hall (1929–1931) are not applicable to all Protozoa.

The question of homologizing these osmiophilic granules with Golgi materials described in other Protozoa and in the Metazoa will be treated fully in the latter part of this paper. For the present, the noncommittal descriptive phrase "osmiophilic granules" will be used to designate them.

OBSERVATIONS ON THE VACUOLAR CYCLE

The vacuolar cycles found in *Epidinium* and *Eudiplodinium* are described in detail, since they show two widely differing types of contractile vacuoles. The cycles found in *Ophryoscolex, Ostracodinium, Polyplastron,* and *Metadinium* are described chiefly in respect to the differences brought out by comparison with *Epidinium* and *Eudiplodinium.*

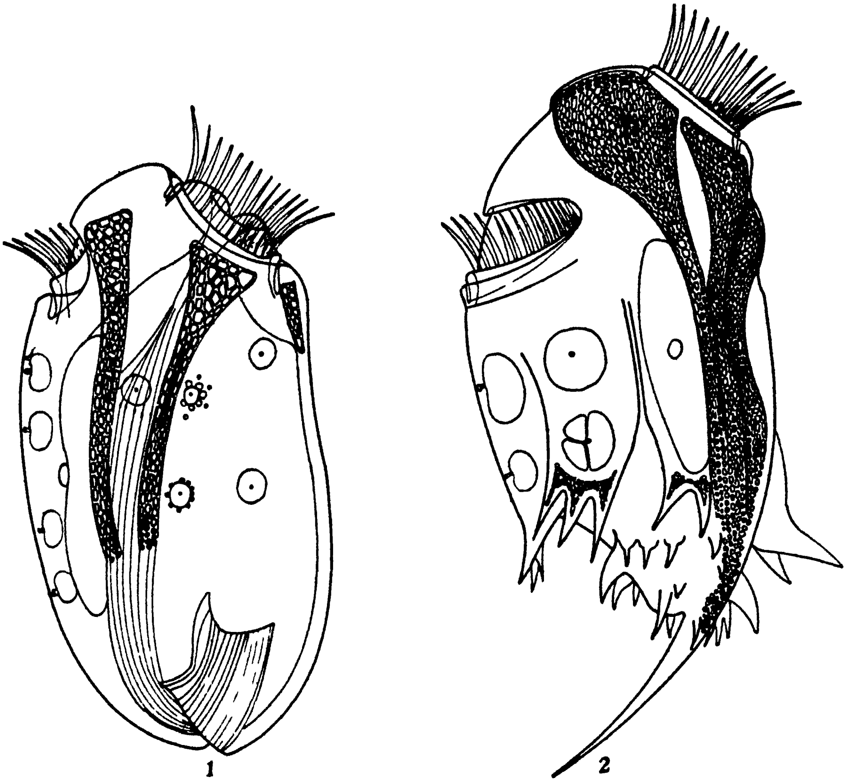

Fig. B. *1, Polyplastron multivesiculatum,* × 500; *2, Ophryoscolex caudatus,* × 500, semidiagrammatic.

CONTRACTILE VACUOLES OF EPIDINIUM

Epidinium caudatum (Fiorentini, 1889) was the commonest species in the material studied. *Epidinium ecaudatum* (Fiorentini, 1889), *E. bicaudatum* (Sharp, 1914), and *E. tricaudatum* (Sharp, 1914) were less common, but sufficient material was available to determine that the vacuolar cycle in these species is identical with that in *E. caudatum.*

Two vacuoles are present in *Epidinium* (fig. A, *3*). They lie along the dorsal mid-line, the anterior vacuole lying just behind the dorsal membranelle zone and the posterior vacuole near the level of the posterior end of the macronucleus. The anterior vacuole is slightly to the right of the posterior vacuole, due to the twisting of the body (Sharp, 1914).

The pore of each vacuole (fig. C, *7*) is a circular opening in the pellicle, from 1 to 2μ in diameter. From it a short tube, the excretory canal, extends inward for a distance of from 2 to 4μ. The wall of the canal is formed by a continuation of the pellicle which covers the body of the ciliate. A thin membrane is stretched over the inner end of the excretory canal, closing it during diastole.

The fully expanded vacuole (fig. C, *1, 7*) is a simple lentoid vesicle flattened dorso-ventrally. The inner surface of the vacuole is convex, while the outer surface is slightly concave where the vacuole is pressed against the stiff excretory canal. In ten specimens the antero-posterior diameter ranged from 12 to 22μ, the dorso-ventral diameter from 8 to 13μ. The anterior vacuole in most specimens is from 2 to 4μ larger in both dimensions than the posterior vacuole. There are also many small accessory vacuoles around the main vacuole at this time. The vacuole in this stage may remain unchanged for a considerable period, which may be referred to as the resting stage.

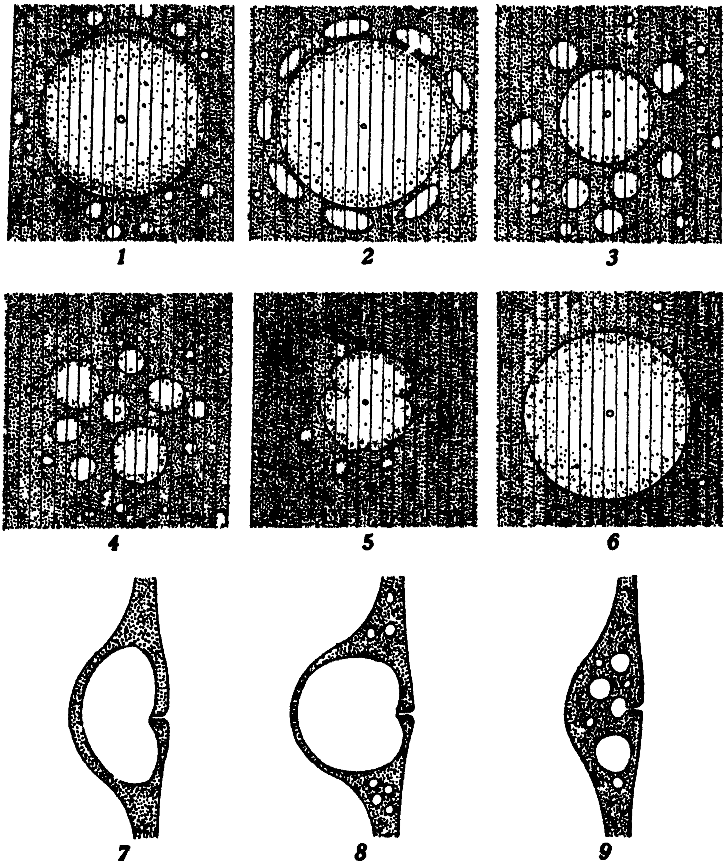

Fig. C. *Epidinium caudatum.* Contractile vacuoles during the pulsatory cycle drawn from live specimens stained vitally with neutral red. Granules stained vitally with neutral red are arbitrarily drawn larger than the non-staining granules. Semidiagrammatic, × 1000. *1–6*, dorsal view of anterior contractile vacuole; *7–9*, lateral view of anterior contractile vacuole in optical section. *1*, resting stage; *2*, late resting stage; *3*, systole; *4*, late systole; *5*, diastole; *6*, end of diastole; *7*, resting stage; *8*, vacuole rounded just before systole; *9*, systole.

Cycle.—The lentoid vesicle described above is surrounded by a region showing little, if any, Brownian movement, indicating a gel state. The first sign of activity is an increase of movement in the surrounding granules, accompanied by a rounding of the vacuole to a spherical shape (fig. C, *8*) slightly indented at one pole where it presses against the canal. This preliminary stage lasts about ten seconds. The vacuole then undergoes a sudden convulsion in adjusting itself completely on the canal. This is evidently the result of a further solation of the surrounding ectoplasm. The membrane sealing the pore ruptures at the same time and systole occurs immediately (fig. C, *4, 9*), lasting from 1.2 to 11.1 seconds and usually taking about 4 seconds. The excretory canal is again sealed by a membrane which is formed from the remnants of the wall of the old vacuole. Systole is usually complete, but from time to time incomplete systole in all gradations was observed. Several successive incomplete systoles were observed in three specimens. In these systoles the small remaining vacuole drew away from the canal and the wall of the vacuole was pulled out in a long string which finally broke. The part attached to the canal forms the closing membrane. The vacuole occasionally becomes spherical, the cytoplasm changing from gel to sol, without starting systole. The cytoplasm then gradually reverts to a gel state. If the sudden, final solation occurs, systole invariably follows.

The cytoplasm around the vacuole streams in during systole (fig. C, *3, 4*) carrying along the small accessory vacuoles. These vacuoles, along with any remnant of the old vacuole, then fuse to form the new vacuole (fig. C, *5*). This quickly comes in contact with the inner end of the canal, becomes attached to it, and remains there for the rest of the vacuolar cycle. The cytoplasm is in a sol state from the rounding up of the vacuole at the end of the resting stage to this early fusion of accessory vacuoles at the beginning of diastole. After this fusion of the accessory vacuoles the cytoplasm around the contractile vacuole reverts to a gel state which is marked by a slowing up and final cessation of Brownian movement and a marked irregularity in the outline of the vacuolar wall. This process of fusion continues for some time while the main vacuole grows to nearly full size. This early period of diastole occupies about one minute from the time of completion of systole, and in this time nearly all the accessory vacuoles become incorporated in the main vacuole (fig. C, *6*). The vacuole then increases in diameter without the addition of any more accessory vacuoles. The growth of the main vacuole gradually ceases and a new ring of accessory vacuoles appears around it. This ring corresponds to the V_2 group in *Euplotes* (Taylor, 1923) and is composed of vesicles which form the next contractile vacuole. The vacuole may remain quiescent in this state for some time, the usual period being about five minutes.

The time occupied by a single vacuolar cycle varied in the specimens observed by the writer from one minute to over an hour. The period of the early stage of diastole, the formation of the vacuole by fusion of the smaller vesicles, is constant, but the second stage, the growth without fusion of accessory vesicles, may be ended at any time by systole.

The small accessory vacuoles originate in all parts of the ectoplasm on the dorsal side of the ciliate. They first appear as minute vacuoles, about one micron in diameter, each with one or two colorless granules and occasionally one or two granules stainable with neutral red adhering to the surface. These small vacuoles migrate slowly toward the region of the main vacuole and at the same time fuse with the small vacuoles. No ectoplasmic currents could be observed which could account for this migration, the vacuoles appearing to force their way among the relatively motionless granules of the cytoplasm.

Relationship to osmiophilic granules.—The main vacuole in the quiescent phase of late diastole (pl. 16, fig. 19) appears in impregnated material as a clear vesicle limited by the yellowish orange wall and cytoplasm. A few blackened granules adhere to the vacuolar wall and others are scattered through the ectoplasm. The small accessory vacuoles surrounding the main vacuole are completely blackened by a swarm of osmiophilic granules. These granules are often so closely spaced that the accessory vacuoles appear to have homogeneous black walls with a rough, granular, outer surface.

No stages which could be interpreted as systole were found. Since systole is so short, this is rather to be expected.

The vacuole in early diastole (pl. 15, fig. 1; pl. 16, fig. 16), is a small vesicle with many black granules covering its surface and small black accessory vacuoles near it or in various stages of fusion with it. The number of osmiophilic granules adhering to the wall gradually diminishes in the larger vacuoles (pl. 16, fig. 17) until a stage is reached in which the vacuole is clear except for a few granules on its surface (pl. 16, fig. 18) and with very few accessory vacuoles grouped around it. This corresponds to the appearance of the living vacuole at the end of the first growth period. From then on, the main vacuole is surrounded by more and more small blackened vacuoles of the V_2 group. The blackened granules, as shown both in whole mounts and in sections (pl. 18, fig. 39) are always outside the vacuole, never inside. The membrane itself is not impregnated, the appearance of impregnation being caused by the close packing of granules on the surface.

The smallest accessory vacuoles observed had only one or two osmiophilic granules adhering to their surfaces. More granules are found around slightly larger vacuoles, until finally, in the accessory vacuoles near the main vesicle, it is difficult to see the clear vesicle in the middle of the aggregation of granules. This accumulation of osmiophilic granules around the accessory vacuoles and around the main vacuole during diastole indicates that these granules are probably closely connected with the concentration of materials within the vacuole.

CONTRACTILE VACUOLES OF EUDIPLODINIUM MAGGII

Two large contractile vacuoles lie beneath the right dorsal surface of *Eudiplodinium maggii* (pl. 15, fig. 8). The outer exretory pore of each vacuole is an ellipsoid from 3 to 8μ long and from 2 to 5μ wide, with its long axis lying parallel to the main axis of the body. These pores open on the right dorsal surface instead of along the dorsal mid-line as in most of the other genera. The excretory canal (fig. D, *7–9*) extends inward from the pore for the considerable distance of from 10 to 15μ and tapers rapidly, so that the inner pore is only from 2 to 4μ in diameter. This canal is formed by an extension of the pel-

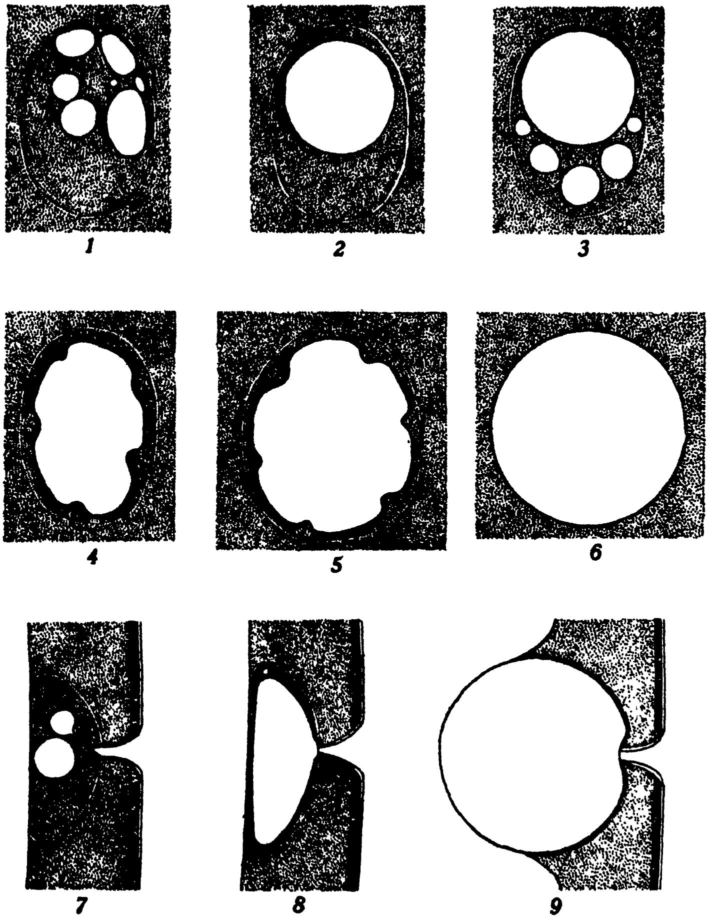

Fig. D. *Eudiplodinium maggii*, × 1000. Anterior contractile vacuole during the pulsatory cycle from a specimen stained vitally with neutral red. Granules staining with neutral red are arbitrarily drawn larger than the non-staining granules. Semidiagrammatic. *1–6*, lateral view in optical section; *7–9*, dorsal view in optical section. *1*, end of systole; *2–3*, first stage of diastole; *4–5*, second stage of diastole; *6*, vacuole rounded just before systole; *7*, end of systole; *8*, end of first stage of diastole; *9*, vacuole rounded just prior to systole.

licle, as in other genera. The pellicle covering the whole body has two distinct layers (pl. 18, figs. 44–46), an inner striated layer (from 1 to 2μ in thickness) and an outer clear layer (less than 0.5μ in thickness). Only the outer layer is continued inward to form the canal, which pierces the striated layer.

The resting vacuole is a large, clear vesicle (pl. 18, fig. 46), slightly elongated along the antero-posterior axis. The length of ten different resting vacuoles ranged from 20 to 30μ, the dorso-ventral diameter from 15 to 22μ. There is no specialized region of cytoplasm around the vacuole at this stage, nor are there any accessory vacuoles in the general ectoplasm. The granules near the vacuolar membrane show only a small amount of Brownian movement.

Cycle.—In preparation for systole the resting vacuole rounds up for a period of from 5 to 8 seconds (fig. D, *6, 9*), following a preliminary lowering of the viscosity of the cytoplasm immediately surrounding the vacuole. Then a further solation takes place, the vacuole completes the rounding up process in a sudden convulsion, and systole immediately follows. Systole takes from 2.1 to 10 seconds and is usually complete, the remnants of the vacuolar wall forming the membrane closing the inner excretory pore. In some specimens systole was arrested after varying amounts of fluid had been expelled. When only a small vacuole was left (fig. D, *7*) this was pulled away from the pore, apparently by the strong currents in the ectoplasm of the vacuolar region. The vacuolar wall was pulled out in a narrow thread which finally broke, one end was resorbed into the wall of the freed vacuole, the other end formed a smooth membrane across the inner pore. In a very short systole only a small amount of fluid is expelled, and the closing membrane is apparently formed by a slight constriction of the vacuolar wall just below the inner pore.

The ectoplasmic matrix carrying with it a host of small non-stainable granules rushes in during systole (fig. D, *1*) behind the retreating wall of the vacuole. The granules stainable with neutral red remain stationary. The general ectoplasmic mass around the vacuole fills in a part of the space formerly occupied by the resting vacuole. Thus at the end of systole there is a definite region beneath the excretory canal somewhat smaller than the resting vacuole and containing a large number of granules which do not stain with neutral red, in violent Brownian movement and also tumbling around as if there were strong but exceedingly localized currents in the clear matrix. The general ectoplasm containing both clear non-staining granules and granules stained with neutral red surrounds this region, and the granules in this region show very little Brownian movement as compared to their movement in the vacuolar region. A very narrow, almost invisible, hyaline layer separates the two regions.

Accessory vacuoles arise in this specialized region even while it is forming and before the old vacuole is completely discharged. These vacuoles tend to arise along the anterior border and often outline it clearly. Accessory vacuoles forming in the middle float around freely and often touch, but show no tendency to fuse until after systole is complete. They then fuse (fig. D, *2*) forming a single, large vacuole almost completely filling the anterior portion of the vacuolar region. More vacuoles form in the remainder of the granular area (fig. D, *3*) and fuse with the first vacuole. This formation of accessory vacuoles and subsequent fusion ceases when only a narrow band of granular material remains around the clear vacuole (fig. D, *4, 8*) and brings to an end the first growth period in from thirty seconds to two minutes after the completion of systole.

Extreme solation of the material within this vacuolar region marked it at the beginning and this continues until after the accessory vacuoles begin to fuse. The Brownian movement and turbulence of the ectoplasm of the vacuolar region then gradually diminishes until at the end of the first growth period the wall of the vacuole is sufficiently gelated to retain traces of the accessory vacuoles in the roughened outline of its walls (fig. D, *4*). Outside of this region, however, the consistency of the cytoplasm, as indicated by Brownian movement, shows no such wide fluctuations.

This vacuole with roughened walls continues to enlarge in all directions but without the formation of any accessory vacuoles (fig. D, *5*). The granular zone gradually becomes thinner, and the outline of the vacuole becomes smoother until the elongate resting vacuole with no specialized granular region around it is formed anew. This second growth period lasts from one to two minutes. The resting stage lasts from fifteen seconds to an indefinite length of time.

The formation of the accessory vacuoles in *Eudiplodinium maggii* is remarkable in that it is strictly limited to a short period during systole and to the first growth stage and also is limited in locality to this small, specialized vacuolar region. No accessory vacuoles are formed at any other time or place. Occasionally small vacuoles do appear in the surrounding cytoplasm in individuals from material kept too long, but no such vesicles formed outside of the vacuolar region were ever observed to contribute to the contractile vacuole in any way. One such vesicle lying adjacent to the anterior vacuole was observed to be unchanged throughout seven consecutive cycles of this vacuole.

The formation of an accessory vacuole is first marked by a gradual diminution of granules in a restricted region and increase of movement in the remaining granules. This lasts from five to ten seconds during which time the granules completely disappear. Then very suddenly a differentiation is observable between the clear central region and the surrounding granular region, and a small accessory vacuole, bounded by a distinct protoplasmic wall, becomes apparent.

Relationship to osmiophilic granules.—The resting vacuole in individuals impregnated with osmic acid is a large, clear vesicle surrounded by golden yellow cytoplasm (pl. 18, fig. 46). Few granules occur either in connection with the vacuole or in the general ectoplasm. The next stage found in fixed material was that just at the completion of systole (pl. 17, fig. 36; pl. 18, fig. 47). The vacuole in this stage is a small vesicle completely filled with thickly packed, heavily blackened granules. A clear central vacuole bounded by a gray reticulum then appears (pl. 15, fig. 8; pl. 17, fig. 34; pl. 18, figs. 44, 48), usually in the anterior portion of the granular mass, and in other later stages more clear vacuoles appear (pl. 18, fig. 49). This vacuolization of a dense, granular osmiophilic region corresponds exactly to the vacuolization of the granular vacuolar region observed in living material.

The absolutely sharp demarkation of locality of granules in *E. maggii* furnishes further evidence that the granules stainable with neutral red are entirely different from the osmiophilic granules and that the two types of granules perform two entirely different functions. The osmiophilic granules are found only within the vacuolar region, while the granules stainable with neutral red are concentrated outside of it and very few, if any, are found within the vacuolar region.

Kraschenninikow (1929) has previously described the results of osmic impregnation in *E. maggii.* He described both the black granules and the gray reticulum. He claims that the vacuolar membrane itself was impregnated, the granular appearance being caused by unevenness in the membrane. The black granular portion and the gray reticulum were homologized with the intensely osmiophilic "apparat externum" and the less intensely osmiophilic "apparat internum" respectively, described by Hirschler (1927) in the Golgi apparatus of various metazoan and protozoan cells. The presence of black granules and a gray reticulum was confirmed in the present investigation, but no evidence was found to support Kraschenninikow's statement that the osmiophilic granules are a part of the vacuolar membrane.

Origin of accessory vacuoles.—The formation of accessory vacuoles in *E. maggii* apparently occurs by the solution of the osmiophilic granules, first in localized regions and finally involving all of them. Sections of impregnated vacuoles in early stage of diastole with accessory vacuoles forming is very suggestive in regard to the rôle of the osmiophilic granules in the formation of accessory vacuoles. These granules are massed in a heavy band around a central cavity (pl. 18, fig. 44). Close examination shows that they are suspended in a fine reticulum, which was turned a dark gray by the action of the osmium tetroxide. This gray reticulum also fills the spaces between small clear vesicles within the granular band. Later stages show a large clear vesicle, evidently formed from a fusion of the smaller, clear vesicles (pl. 18, fig. 45) and by the reduction and finally the disappearance of the granular band.

The evidence for the solution of the osmiophilic granules is much more convincing if certain phenomena observed in living material are considered along with the evidence from fixed material. In systole the migration of a large number of granules into the vacuolar region is easily observed, but in diastole no movement outward could be observed, although the granules unmistakably disappear and are replaced by the clear, watery, vacuolar fluid. The direct observation of the solution of granules does not seem to be feasible owing to their large number and incessant activity which make it impossible to follow continuously any one granule or set of granules. The observation nearest to direct proof of solution is that, just prior to the formation of any particularly accessory vacuole, the number of granules in that region is greatly reduced and they disappear completely as the accessory vacuole is finally formed. Therefore, from a consideration of both living and fixed material, the conclusion that the granules dissolve in the vacuolar fluid seems unavoidable.

CONTRACTILE VACUOLES OF OPHRYOSCOLEX CAUDATUS

Ophryoscolex caudatus Eberlein 1895 (fig. B, *2*) possesses nine contractile vacuoles arranged in four longitudinal pairs around the middle portion of the body, with the ninth vacuole in the portion just ventral to the skeletal plate. Each vacuole opens to the surface by a short, cylindrical excretory canal from 2 to 4μ in length and from 1 to 2μ in diameter. The canal is formed by an inward extension of the pellicle, which is a single homogeneous layer as in *Ostracodinium, Epidinium,* and *Polyplastron.*

Cycle.—In general, the vacuolar cycle of *Ophryoscolex* is similar to that in Epidinium. The resting vacuole (fig. E, *1*) is a large, lentoid vesicle from 20 to 40μ in the antero-posterior diameter and from 15 to 25μ thick. Systole is relatively rapid, lasting from 0.4 to 1.9 seconds. A small vacuole always remains after systole (fig. E, *3*). The fusion of the new accessory vacuoles takes place from 30 to 50 seconds after the end of systole, the rest of diastole lasting from 1 to 5 minutes. The resting period in *Ophryoscolex* is unusually long, normally lasting from 15 to 45 minutes. Occasionally the complete cycle lasts only two minutes.

The osmiophilic granules of *Ophryoscolex,* while common in the general ectoplasm, never show so marked a degree of concentration around the vacuoles as do those of *Epidinium.* This may be correlated with the relative slowness of the vacuolar cycle in *Ophryoscolex* and with the infrequency of concentration of any one vacuole, as well as with the relatively widespread formation of the accessory vacuoles in the ectoplasm. Since the osmiophilic granules are primarily concerned with formation of the accessory vacuoles, the osmiophilic granules also are scattered rather than concentrated near the contractile vacuole.

CONTRACTILE VACUOLES OF OSTRACODINIUM

Ostracodinium monolobum Dogiel 1927 was the species most thoroughly studied in this investigation. *O. gracile* (Dogiel, 1925) and *O. mammosum* (Railliet, 1890) were also investigated as a check on the results obtained from *O. monolobum*.

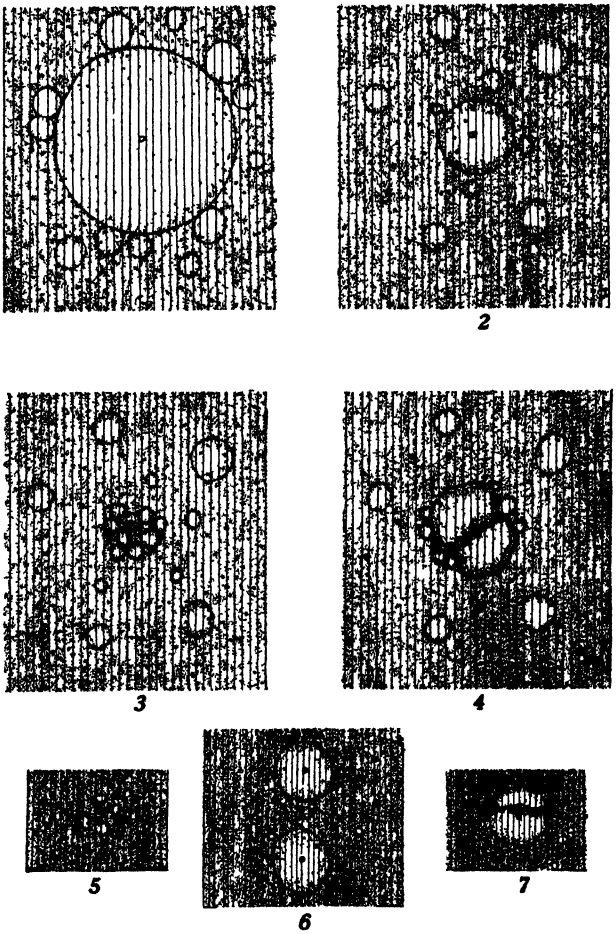

Fig. E Surface view of contractile vacuoles during the pulsatory cycle from individuals stained vitally with neutral red. Granules staining with neutral red are arbitrarily drawn larger than the non-staining granules. Semidiagrammatic, × 1000.

1–4, Ophryoscolex caudatus. 1, resting stage; *2,* systole; *3,* end of systole; *4,* diastole.
5–7, Ostracodinium monolobum. 5, end of systole; *6,* resting stage; *7,* diastole.

There are from two to six contractile vacuoles along the dorsal mid-line of *Ostracodinium.* Two vacuoles are present in *O. gracile,* three in *O. mammosum,* and five in *O. monolobum* (pl. 15, fig. 5). These vacuoles are adjacent to the left border of the macronucleus, lying in a longitudinal strip of ectoplasm thicker than that of the rest of the body. Fully expanded vacuoles vary in antero-posterior diameter from 4 to 20μ and in dorso-ventral diameter from 3 to 12μ. In species with a relatively large number of vacuoles such as *O. monolobum,* the region of gel around the vacuoles forms a continuous longitudinal strip in the general ectoplasmic sol. This strip is also marked by a concentration of neutral red granules and Golgi granules.

Cycle.—The preliminary rounding of the vacuoles lasts from 8 to 10 seconds, while systole only lasts from 0.8 to 1.9 seconds. The main vacuole contracts until a vesicle about 4μ in diameter remains (fig. E, *5*), and then systole ceases. The neutral red granules retain their position for a short time after systole, so that the former position of the vacuole is marked for a short time by a sphere of colorless, granular ectoplasm surrounded by ectoplasm filled with small accessory vacuoles and with granules stainable with neutral red. The whole vacuolar cycle lasts at least two minutes, the usual period for an actively contracting vacuole being about 2.5 minutes.

The accessory vacuoles in most of the Ophryoscolecidae are formed in a more or less limited region around a particular vacuole and fuse only with it. The vacuoles in *Ostracodinium monolobum,* on the other hand, are so close together that the accessory vacuoles may fuse with either one of two adjacent contractile vacuoles. In some specimens the accessory vacuoles assembled near a canal after systole fuse with the adjacent contractile vacuole instead of forming a new vacuole for the canal. In other specimens two contractile vacuoles may grow so large that they touch and fuse, the double vacuole then becoming detached from one of the canals and emptying through the other. In such an occurrence the only indication of one vacuole is the canal and pore. These variations produced by the extreme crowding of the contractile vacuoles, and also the occasional formation of large accessory vacuoles which may be confused with the true contractile vacuoles, make the correct counting of the contractile vacuoles in fixed specimens of the species difficult and at times impossible.

A layer of osmiophilic granules is found around the vacuole during diastole and shows clearly the formation of accessory vacuoles within it (pl. 17, fig. 35). This vacuole is strikingly similar to the vacuole of *Cycloposthium dentatum* f. *scutigerum* described by Strelkow (1931*a,* pl. 15, figs. 19–20). He interpreted this as a stage in the division of the vacuole. The individual *O. obtosum* from which our figure was made, showed no indication of the multiplication of any organelles, which marks division; also this stage in fixed material corresponds to the stage of diastole in which growth of the vacuole is accomplished by the formation and fusion of small accessory vacuoles as observed in living material. We conclude that his interpretation is incorrect. The appearance of small vesicles in the osmiophilic region around a contractile vacuole indicates the formation of accessory vacuoles rather than a division of the whole organelle.

CONTRACTILE VACUOLES OF POLYPLASTRON MULTIVESICULATUM

Polyplastron multivesiculatum (Dogiel and Fedorowa, 1925) shows as many as ten vacuoles (fig. B, *1*), a larger number than any of the other Ophryoscolecidae. Four vacuoles are arranged longitudinally along the dorsal mid-line. One vacuole lies just to the left of the anterior vacuole of the dorsal row. The rest of the vacuoles are found under the right side. One is occasionally found between the two main skeletal plates. One pair of vacuoles, arranged longitudinally, lies ventral to the right skeletal plates, and another pair is placed parallel to the first pair and ventral to it. The number of vacuoles present in an individual varies considerably from time to time. Only seven vacuoles were present in one individual with a retracted oral zone. The oral zone opened shortly after the observations were started and the ciliate began to feed. Four more vacuoles appeared in a short time, each of them going through several pulsatory cycles. Pores and excretory canals were found in individuals with less than ten vacuoles in the positions occupied by vacuoles when all were present. These observations were repeated on many other individuals. The variation in the number of vacuoles of *Polyplastron* is dependent, therefore, upon the intake of fluid during feeding. During periods of lessened fluid intake, some of the vacuoles are indicated only by the excretory pores and canals.

The fully expanded vacuole (fig. F, *5*) is relatively small, with an average diameter of about 15μ (ranging from 12 to 30μ), and a dorso-ventral diameter of from 8 to 15μ. These fully formed vacuoles are smaller and more nearly spherical than those of any other genera studied excepting *Ostracodinium*.

Cycle.—The preliminary rounding of the vacuole lasts from 1 to 3 seconds. Systole then starts, lasting from 1.5 to 3.0 seconds, and is usually complete. The accessory vacuoles and the spherules staining with neutral red retain their position during systole (fig. F, *1, 6*), the space formerly occupied by the resting vacuole being filled by the matrix and the non-stainable spherules.

The fusion of accessory vacuoles is complete in from 20 to 60 seconds. The vacuole increases in size for a time without addition of accessory vacuoles, the wall becomes smooth and the vacuole usually becomes elongated in the antero-posterior axis. A concentrated ring of granules assembles at this time around the vacuole (fig. F, *4*) and small accessory vacuoles arise within it and empty into the main vacuole. The spherules forming this ring are not stainable with neutral red. The ring of spherules gradually disappears as the accessory vacuoles form within it. Some of these vacuoles remain intact and later fuse to form the next contractile vacuole. The formation and disappearance of the ring occupies from 30 to 60 seconds.

The total cycle from systole to systole takes from fifty seconds to ten minutes, the average time in the specimens studied being about two minutes.

Most of the accessory vacuoles arise just after systole, replacing the old contractile vacuole. The granules in parts of this region gradually decrease in number and then very suddenly the clear vacuolar fluid is differentiated from the surrounding ectoplasm, which becomes more viscous. Smaller solated regions appear also in the surrounding ectoplasmic gel and small accessory vacuoles are formed in the same manner. All these vacuoles migrate toward the canal and fuse to form the small, irregular vesicle of early diastole. The formation of the small accessory vacuoles in the granular ring in late diastole is not so apparent as that described above as occurring just after systole.

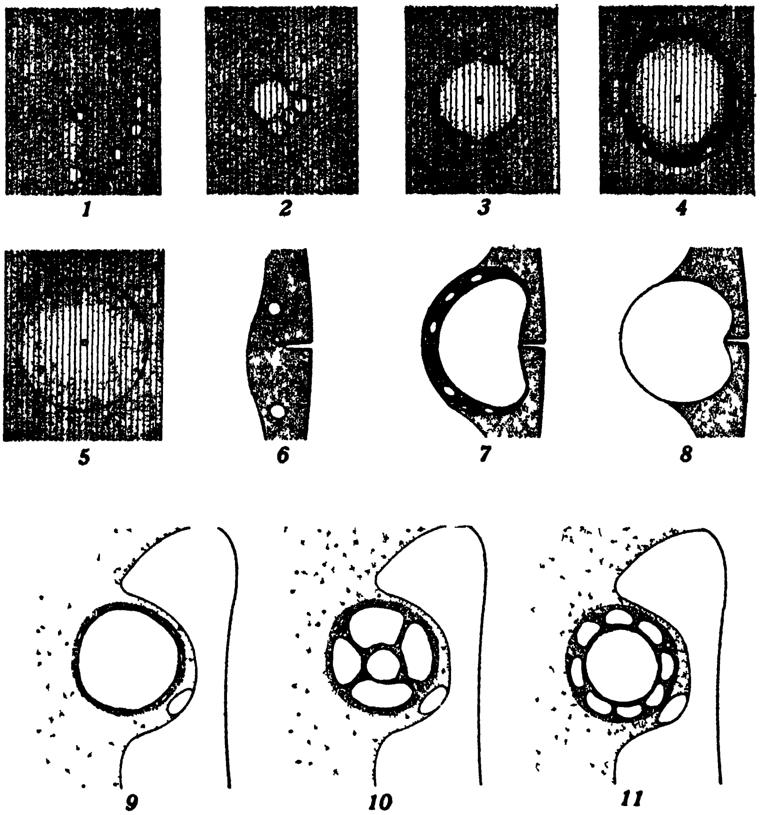

Fig. F. Contractile vacuoles during the pulsatory cycle from live specimens stained vitally with neutral red. Granules staining with neutral red are arbitrarily drawn larger than the non-staining granules. Semi-diagrammatic.

1–8, *Polyplastron multivesiculatum*, × 1000. *1–5*, surface view of anterior dorsal vacuole; *6–8*, side view of the same vacuole in optical section. *1*, end of systole; *2*, beginning of diastole; *3*, end of the first stage of diastole; *4*, second period of diastole; *5*, resting stage; *6*, end of systole; *7*, second stage of diastole; *8*, vacuole rounded just prior to systole.

9–11, *Metadinium medium*, × 500, lateral view of the anterior contractile vacuole in optical section. The anterior end of the macronucleus and the micronucleus are shown in outline. *9*, resting stage; *10*, middle of systole; *11*, late diastole.

At the end of systole, as shown by osmic preparations, the vesicle is replaced by several small vacuoles (pl. 17, fig. 20), around which are clustered a number of relatively large osmiophilic granules. In the next stage, the single small vacuole of early diastole (pl. 17, fig. 21) is surrounded by a layer of very fine osmiophilic granules in which are imbedded a few large granules such as occur in the previous stage. As the accessory vacuoles fuse, these large granules either break up and form this band of smaller granules, or they disappear and are replaced by a new set of small ones. The origin of the smaller granules from the larger ones seems the more reasonable interpretation. This osmiophilic band thickens (pl. 17, figs. 22, 23; pl. 15, fig. 7) and in some specimens reaches a diameter of 5μ. The granular character of this band is apparent only in material impregnated at room temperature (pl. 17, fig. 25). In material impregnated with osmium tetroxide at from 30° to 35° C (pl. 17, fig. 26), the granular zone appears as a homogeneous black band, the only evidence of the original granular structure being the rough outer surface.

In the next stage, small clear vesicles are found in the osmiophilic region (pl. 17, fig. 27). Since this band of osmiophilic granules corresponds exactly to the band of closely packed granules observed during the vacuolar cycle in the live specimens, it is clear that the osmiophilic granules and the granules stainable with neutral red are two entirely different types of cytoplasmic inclusions.

CONTRACTILE VACUOLES OF METADINIUM MEDIUM

Metadinium medium Averinzew and Mutafowa 1914, the only species of the genus found thus far in California cattle, possesses two large, nearly spherical vacuoles beneath the right dorsal surface (fig. A, *2*). These vacuoles are half imbedded in two large dorsal hollows of the macronucleus, one near the anterior end and one near the posterior end.

The excretory canal is from 8 to 10μ long (pl. 17, fig. 32) and opens by means of a small, circular, outer excretory pore, from 3 to 5μ in diameter. The pellicle of *Metadinium,* as well as that of *Eudiplodinium,* is in two layers, a thick inner striated layer, and a thin, outer clear layer. An inward extension of the clear layer alone forms the wall of the excretory canal.

The resting vacuoles (fig. F, *9*) of *Metadinium medium* are large and nearly perfect spheres, from 25 to 35μ in diameter. A narrow, densely granular rim from 1 to 3μ in thickness surrounds each vacuole. Small flattened accessory vacuoles are imbedded in this region. A few granules stainable with neutral red are found in the rim, but the majority of the granules could not be stained with any of the vital dyes used in this investigation. None of the granules in the vacuolar region exhibit Brownian movement at this time, demonstrating that it is a relatively stiff gel. The general ectoplasm has fewer granules than the vacuolar region, but a large proportion of these granules are stainable with neutral red, so that in an individual stained with this dye the pale greenish gray vacuolar region is bounded by myriads of brilliant red granules. The granules in the general ectoplasm show marked Brownian movement, the sol state thus indicated differing markedly from the gel state of the granular band around the vacuoles.

Cycle.—The main vacuole contracts in a leisurely fashion (fig. F, *10*), taking from 21.2 to 26.0 seconds for the completion of systole. The small accessory vacuoles in the granular rim expand rapidly and fuse together as the main vacuole contracts. There is at the same time a definite flow of granules from the general cytoplasm into the vacuolar region, so that the total diameter of the vacuolar region (vacuole plus granular rim) remains unchanged. Systole is usually complete and the remainder of the wall of the old vacuole forms the closure membrane over the inner excretory pore.

The osmiophilic granules in *Metadinium* form a heavy band around the contractile vacuole and are also scattered evenly throughout the whole ectoplasm. Only the granules in the band around the vacuole appear to be concerned directly with the vacuolar cycle. The osmiophilic rim can be demonstrated in osmic preparations in all stages of the cycle. This confirms the permanency of the granular rim as observed in living specimens and emphasizes the contrast to the granular region, which is present in *Eudiplodinium* only in diastole.

STRUCTURE OF THE CONTRACTILE VACUOLE IN THE OPHRYOSCOLECIDAE

Pore.—The presence of a permanent pellicular pore has been denied for many ciliates. Khainsky (1910) described instead a rhythmic rise and fall of the pellicle over the vacuole of *Paramecium* during the pulsatory cycle and gave the name "papilla pulsatoria" to the portion of the pellicle involved. This activity was described entirely from fixed material. Haye (1930) described a similar appearance in *Polyplastron multivesiculatum* and *Ostracodinium gracile* and like Khainsky found no excretory pores or canals. The observations reported in this paper, however, show that the papilla pulsatoria is never present in living material and that the rise and fall of the pellicle over the vacuole as figured by Haye is caused by the processes of fixation, imbedding, and sectioning.

The papilla pulsatoria described by Taylor (1923) in *Euplotes* is a thin protoplasmic membrane sealing a permanent pore in the hard, rigid pellicle. The drawings published by Bretschneider and Hirsch (1927) of the papilla pulsatoria in *Balantidium giganteum* indicate a similar type of structure. These structures are entirely different from the large invaginations and evaginations of the pellicle of *Paramecium* described by Khainsky and should not be included under his term.

The excretory canal and the inner and outer excretory pores are permanent pellicular structures in the Ophryoscolecidae. The canal is rigid at all times and shows no traces of sphincters like those described by Strelkow (1931*a*) in the Cycloposthiidae. The inner pore is closed at all times, except during systole, by a thin protoplasmic membrane, formed from the remnants of the wall of the old vacuole. The inner excretory pore of the contractile vacuoles in the Ophryoscolecidae is similar to the permanent opening in the pellicle of *Euplotes,* sealed except during systole by a delicate protoplasmic membrane. Zenker (1866) gave a similar interpretation for the vacuole of several ciliates.

Vacuolar wall.—The presence of a permanent membrane bounding the contractile vacuole has been the subject of much dispute, but since the observations of Wrzesniowski (1869) it has been generally admitted that in those vacuoles which are formed by the fusion of newly arising accessory vacuoles, the boundary membrane is only a temporary differentiation of the cytoplasm. Taylor (1923), on the basis of his observations on *Euplotes,* corroborates this view.

The wall surrounding the contractile vacuole and the accessory vacuoles of the Ophryoscolecidae is temporary, disappearing during systole and re-forming anew as each new accessory vacuole is formed. The wall, like that in *Euplotes,* is not a membrane in the structural sense, since only the internal surface of the wall is sharply differentiated and the outer surface grades indistinctly into the surrounding ectoplasm.

Kraschenninikow (1929) and Haye (1930) described a definite membrane around the contractile vacuoles in several genera of the Ophryoscolecidae. Kraschenninikow claims that the membrane is osmiophilic. Haye described small granules imbedded in the membrane and stated that these are lipoidal in nature. He also described the accessory vacuoles as arising in this membrane, appearing in his drawings as little blisters. The presence of a definite membrane in the vacuoles of the Ophryoscolecidae is due entirely to fixation and is the result of partial fusion of the granules immediately surrounding the vacuole, giving the appearance of a heavy, vacuolated membrane. In *Eudiplodinium,* the form on which Kraschenninikow based his conclusions, the boundaries of the vacuoles are relatively inconspicuous in material impregnated with osmium tetroxide, while the outer boundary of the vacuolar region is very sharp. Thus Kraschenninikow mistook the outer limit of the heavy granular vacuolar region surrounding the vacuole proper for the vacuolar membrane.

The consistency of the cytoplasm forming the vacuolar wall is usually described as being slightly more viscous than the adjacent cytoplasm (Taylor, 1923; Howland, 1924). This conclusion is based both on micro-dissection experiments and on the fact that the granules next to the vacuole do not show Brownian movement. A similar narrow rim of viscous material adhering to the vacuole is present in the Ophryoscolecidae.

The viscous layer apparently solates at times and allows the accessory vacuoles to fuse. The ability of accessory vacuoles to fuse is dependent on the phase of the vacuolar cycle. Some of the accessory vacuoles formed in the extremely fluid vacuolar region of *Eudiplodinium* during the end of systole bump together quite forcibly under the influence of the localized cytoplasmic currents in this region but show absolutely no tendency to fuse at this time. After systole is entirely completed, however, these accessory vacuoles fuse with one another at the slightest touch. Howland (1924) notes a similar phenomenon in *Amoeba.* Therefore, although the immediate cause of the fusion of two contiguous vacuoles is their contact, contact alone is insufficient to cause fusion unless another factor is operative. This necessary factor is probably the solation of the delicate, indeterminate gel envelope forming the vacuolar wall.

Vacuolar region.—Differentiations of cytoplasm around the contractile vacuoles have been noted in many ciliates. Fauré-Fremiet (1925), Fortner (1926), and others have considered this region to be intimately concerned with the phenomena of vacuolar cycles and have called it the "Nephridialplasma." *Spirostomum* (Day, 1930; Haye, 1930), and *Blepharisma* (Haye, 1930), etc., show no specialized region around the contractile vacuoles.

The position of the main vacuoles is permanent in all the Ophryoscolecidae and the formation of accessory vacuoles takes place in a more or less limited area, the degree of localization varying in the different genera. No region of cytoplasm around the vacuole is visibly differentiated from the rest of the ectoplasm in either *Ophryoscolex* or *Epidinium.* The vacuoles of *Ostracodinium* lie in a longitudinal band of gelated ectoplasm, solation occurring only in localized regions around an active vacuole. There is a temporary differentiation of ectoplasm around the vacuoles of *Polyplastron* during diastole. A very definite band of ectoplasm forms around the vacuoles of *Eudiplodinium* during diastole. In *Metadinium* a similar band is present during the whole cycle. The formation of the new vacuoles in both of these genera is entirely restricted to this vacuolar region. The material of these specialized regions is clearly derived from the general ectoplasmic material and in particular represents a concentration of osmiophilic granules. Thus within the Ophryoscolecidae all degrees of concentration of vacuolar materials are represented and the genera may be arranged in an intergrading series extending from *Ophryoscolex* with a very slight concentration of osmiophilic granules around its vacuoles during diastole, up to *Metadinium* with a permanent band of osmiophilic granules around its vacuoles. This series also represents a gradation in the formation of accessory vacuoles. These vesicles in *Ophryo-*

scolex form in most of the ectoplasm and arise as very minute vesicles below the range of visibility and can be seen only when fully formed. In *Epidinium* the formation of accessory vacuoles is restricted to the dorsal side; in *Ostracodinium* the formation is restricted to a narrow dorsal strip and the vesicles are visible from their earliest stages; in *Polyplastron* they form in a similar restricted region, and in later diastole the last of the accessory vacuoles form in a granular band around the vacuole; finally in *Eudiplodinium* and *Metadinium* they form only in the restricted granular region and are relatively large even in their earliest stages. Thus where there is no localization of formation of accessory vacuoles, there is no concentration of granules around the contractile vacuoles; where there is a localization of vacuolar formation there is a great concentration of granules in the vacuolar region. This correlation of osmiophilic granules and accessory vacuole formation definitely indicates that these granules play an important rôle in the vacuolar cycle.

ORIGIN OF THE ACCESSORY VACUOLES

The differentiation of small accessory vacuoles from the cytoplasm is one of the fundamental problems in a study of the contractile vacuoles. The origin of the accessory vacuoles has been described in other ciliates either as a fusion of pre-existing, but invisible, vacuoles of the ectoplasm, or as an entirely new concentration of fluid with a subsequent differentiation of the vesicle from the cytoplasm. Taylor (1923) showed that the granules of *Euplotes* oscillate in small, fluid-filled vacuoles. He states that in some the granules may dissolve and thus give rise to the small, clear accessory vacuoles (his group V_3). He also shows the possibility of a *de novo* origin.

The accessory vacuoles in *Metadinium, Polyplastron, Eudiplodinium,* and *Ostracodinium* arise as relatively large vesicles and can be made out clearly in all stages of formation. There is first a localized solation of the ectoplasm, then a gradual disappearance of granules in the solated portion, and finally a very sudden differentiation between the clear vacuolar fluid and the surrounding ectoplasm, producing the appearance of a miniature explosion as referred to by Day (1927). There is strong evidence for the solution of the osmiophilic granules in the vacuolar fluid at this time, particularly in *Eudiplodinium* and *Metadinium.* The evidence has been summarized in connection with the description of the osmiophilic granules of *Eudiplodinium.* The accessory vacuoles in these forms do not arise by fusion of ultramicroscopic alveoli but arise *de novo* as relatively large vesicles which are first visible as solated portions of the ectoplasmic gel, the differentiation of the vacuolar contents from the protoplasm being only the end of the whole process of formation. The accessory vacuoles of *Epidinium* and *Ophryoscolex* form as minute submicroscopic vesicles and therefore their method of origin cannot be directly demonstrated. There is, however, no evidence of an alveolar structure of the ectoplasm in these two genera, and since the *de novo* origin is certain in the other four genera investigated, a like origin of the accessory vacuoles in *Epidinium* and *Ophryoscolex* is probable.

The accessory vacuoles normally form in a regular manner, fuse to form one of the contractile vacuoles, and their fluid is thus forced outside of the ciliate. Abnormal specimens, either kept on a slide too long or injured in other ways, show the formation of many irregular vacuoles in all regions of the ectoplasm and in such numbers that only a few of them are eliminated by fusion with the contractile vacuole. Vacuolation is a fundamental property common to all the ectoplasm, but its action is restricted in normal individuals to a relatively narrow region. Thus the formation of a fluid center which results in the localized accumulation of fluid in a vacuole (Dixon, 1920; Taylor, 1923) is accomplished through the action of a specialized region of the ectoplasm and in that sense the contractile vacuolar system is a permanent organelle.

SOURCE OF THE VACUOLAR FLUID

The path of the water through the protozoan cell has excited little interest compared to the attention paid the contractile vacuole itself. Hartog's theory (1888) involves the assumption that the whole surface membrane is permeable to the water of the surrounding medium; also that the intake of water of any given specimen is solely dependent on the concentration of this medium. Degen (1905) and others have accepted his view. Recently the permeability of the whole outer membrane has been questioned to some extent. Taylor (1923) showed that the accessory vacuoles most commonly arose near food vacuoles and on this basis considered the main point of entry to be through the oral region. Eisenberg (1925) considered that in *Paramecium caudatum* most of the water entered by way of the protoplasm bordering the peristome.

There is definite evidence that the pellicle of the Ophryoscolecidae is relatively impermeable. The penetration of vital dyes is very rapid during active feeding, the maximum depth of staining being reached in less than half a minute. When the oral region of one of the Ophryoscolecidae is closed, more than half an hour elapses before its cytoplasmic granules are distinctly colored. Pulsation of any of the vacuoles in a specimen with a closed oral region is rare, but is common in actively feeding specimens. In *Polyplastron* several of the vacuoles are present only when the individual is actively feeding. It is clear that very little of the excess water and other materials can be eliminated through the general surface of the body. It is easily shown by direct observation that very little, if any, of the extra fluid taken in during feeding leaves through the anal opening.

All this evidence indicates that the pellicle covering the general surface of the body is relatively impermeable. The contractile vacuoles alone eliminate most of the fluids. Most of the fluid excreted by the contractile vacuoles enters through the cytostome. One function of the contractile vacuole, therefore, is to eliminate the excess water taken in with the food materials. At the same time, this water, as it is eliminated, serves as the vehicle for dissolved materials such as katabolic wastes.

VACUOLAR CYCLE

Resting stage.—Many contractile vacuoles after attaining full size show a more or less marked period in which no appreciable change either in size or form occurs. This stage is particularly prominent in the Ophryoscolecidae, in which the contractile vacuoles are only occasionally active. Any assemblage of osmiophilic granules is completely absent or at a minimum at this time and the surrounding ectoplasm is firmly gelated. This period of the pulsatory cycle thus presents a marked contrast to diastole and systole which are both accompanied by so many striking cytoplasmic changes. The lack of concentration of osmiophilic granules near the contractile vacuoles during the inactive part of the pulsatory cycle is the condition to be expected if these granules are intimately concerned with the formation of contractile vacuoles.

Systole.—The stimulus for systole is unknown. Taylor (1923) showed that the vacuole of *Euplotes* must touch the excretory pore before systole can occur and concluded that this is the stimulus for systole. The vacuoles in the Ophryoscolecidae, however, are in close contact with the pore during the latter half of diastole and during the resting period. Contact of the vacuole and the pore is therefore not a stimulus for systole in the Ophryoscolecidae. It is possible that in *Euplotes* the correlation between contact and systole is only a coincidence. The size of the vacuoles has nothing to do with commencement of systole. Systole may take place at any time after the first fusion of the accessory vacuoles and a resting vacuole, which thereupon suddenly contracts without any increase in size. Botsford (1926) and others have made similar observations in other Protozoa. Therefore while it has been shown definitely in several forms that systole is immediately preceded by a solation of the membrane closing the excretory pore and of the cytoplasm immediately around the vacuole, the stimulus behind that phenomenon is still unknown.

Many theoretical explanations of systole have been advanced, but some of them are contrary to observed facts and none accounts completely for all the observable phenomena. It is also probable, if not certain, that no single explanation can be applied to the vacuoles of all Protozoa. For example, Dixon's theory (1920) of the contractile vacuole assumes that there is a gel structure surrounding the contractile vacuole throughout the whole cycle and that the energy necessary for the expulsion of the vacuolar fluid comes from the elasticity of this postulated gel. Actually, systole of the contractile vacuoles of many of the Protozoa is accompanied by signs of marked solation of the surrounding cytoplasm. Taylor (1923) showed that the membrane closing the excretory pore in *Euplotes* (his papilla pulsatoria) reverts to a sol condition and ruptures at the beginning of systole, and he implies a solation of the whole region around the vacuole.

Systole of the contractile vacuoles in the Ophryoscolecidae occurs only when the surrounding region is solated. During this solation, the membrane closing the inner excretory pore is dissolved or so weakened that it ruptures. This is exactly opposite to the conditions postulated by Dixon (1920) in his theory of the mechanism of vacuolar action, and his theory cannot be applied either to the Ophryoscolecidae or to *Euplotes.*

The problem of systole is further complicated by the occasional occurrence of partial systole. This is a widespread phenomenon and has been reported in *Euplotes* by Taylor (1923), in various species of *Amoeba* by Botsford (1926), Howland (1924), Howland and Pollack (1927), and in *Actinosphaerium* by Howland and Pollack (1930). Howland and Pollack present evidence to show that partial systole in *Amoeba dubia* is caused by strong cytoplasmic currents which pull the vacuole away from the temporary pore. This was observed in the present investigation in *Ophryoscolex, Ostracodinium, Polyplastron,* and *Eudiplodinium.* Many incomplete systoles were observed, however, in which the remnant of the contractile vacuole remained attached to the excretory pore. In the latter situation, the most we can say is that the forces which produce systole cease to be effective.

The force utilized in expulsion of liquid from the vacuoles is usually ascribed to surface tension, or intracytoplasmic pressure, or both. The theory of systole by intracytoplasmic pressure has been attacked at various times. The chief objection has been that in forms with more than one vacuole, systoles of the vacuoles are not synchronous, although the intracytoplasmic pressure is presumably the same around all the vacuoles. This objection is not valid in the Ophryoscolecidae and probably not in other forms, since the pore of one vacuole may be open while the pores of the other vacuoles are securely plugged by the gelated protoplasmic membrane. The forces of surface tension and of intracytoplasmic pressure are held in check except during systole by the closing membrane.

Diastole.—The diastole of the contractile vacuole is usually considered to involve first an accumulation of osmotically active materials, soluble crystalloid materials, according to Dixon (1920), or katabolic products, according to Taylor (1923), followed by a migration of water to this region. Nassonow (1924) put forth the hypothesis that the osmiophilic region around the vacuoles of certain ciliates is responsible for the accumulation of this osmotically active material. He homologizes the action of this osmiophilic material with that of the Golgi material of secretory cells. Bojewa-Petruschewskaja (1928) showed that the osmiophilic layer around the vacuole of *Balantidium* is demonstrable only during diastole.

Diastole in all genera of the Ophryoscolecidae is in two parts—first the formation and fusion of the accessory vacuoles, next the slow growth of the vacuole to full size in which no more accessory vacuoles fuse with the main vesicle. The cytoplasm immediately around the accessory vacuoles becomes more and more viscous during the fusion process and by the time the second stage of diastole appears it is completely gelated. The osmiophilic granules appear in greatest number in this first period of diastole and then rapidly diminish in number in the second period.

The two parts of diastole demonstrated morphologically in the contractile vacuoles of the Ophryoscolecidae correspond to the two parts of diastole as postulated by present theories of vacuolar action. The early formation of accessory vacuoles and the accumulation of osmiophilic granules represent the localization of osmotically active materials, the passive growth of the vacuole, and the loss of osmiophilic granules in late diastole corresponding to the accumulation of water brought about by the osmotically active materials.

STRUCTURE OF THE ECTOPLASM

The ectoplasm is composed of a clear, optically homogeneous matrix in which many small granules are imbedded. Most of the ectoplasm in the genera observed is normally in a sol state at all times, but the ectoplasm which lies near the contractile vacuoles undergoes periodic sol-gel changes. From direct observation the gel appears to be of the same consistency throughout, but the observed movement of granules during systole cannot be explained on this basis. There is a distinct flow of granules into the vacuolar region of *Eudiplodinium* as the vacuole contracts, and these granules are of one type only, the osmiophilic granules which cannot be stained vitally with neutral red. The granules come from the surrounding gelated ectoplasm in which they are intermingled with the granules staining with neutral red. Thus the non-stainable granules migrate into the vacuolar region while neutral red granules lying next to them remain fixed. A similar differentiation of movements between the two types of granules is found in *Ostracodinium* and *Polyplastron.* The movements of small accessory vacuoles in these latter genera are similar to the movements of the granules. Some of the accessory vacuoles migrate toward the accessory canal shortly after systole while adjacent vacuoles show no movement at all.

These observed differences in the movements of adjacent granules or of adjacent accessory vacuoles may be explained as *resulting from the presence of a* coarse, viscous reticulum in which are imbedded the granules of the vacuome and some of the accessory vacuoles, the interstices between the meshwork being filled with fluid of relatively low viscosity which contains part or all of the granules that do not react to neutral red and also some of the accessory vacuoles. Thus the non-stainable granules are free to move along with the fluid matrix while the neutral red granules are held by the viscous reticulum. This meshwork is relatively coarse and the pores must be at least 0.2μ in diameter in order to allow the granules to migrate freely through them. The reticulum is much larger than the postulated brush-heap structure of a gel and is a structure formed by the gel rather than being the structure of the gel itself.

A viscous reticulum as described above fits in with the observed vacuolar cycles. The ectoplasm around the resting vacuoles of *Ostracodinium* is apparently a solid gel showing no movements of the granules. Just before systole the granules of this region begin to show Brownian movement which is particularly marked in the osmiophilic granules. This marks the first appearance of the coarse viscous mesh and the less viscous phase containing the osmiophilic granules. The homogeneous gel around the resting vacuole thus changes to a viscous reticulum containing a solated phase in its meshes sufficiently pliable to allow the vacuole to become spherical. As the vacuole contracts, the fluid phase fills the space left by the retreating walls of the contractile vacuole. The granules stainable with neutral red thus remain outside the completely solated vacuolar region for a time while the other granules migrate in. Shortly after systole is complete, the neutral red granules also move into the completely solated vacuolar region, indicating that the gelated mesh has also become solated. This interpretation applies also to *Polyplastron*. The gelated reticulum around the vacuoles of *Eudiplodinium* is apparently more permanent, since neutral red granules never get into the vacuolar region. The movements of the granules indicate its presence throughout systole and during the first part of diastole. During late diastole and the resting stage, lack of Brownian movement of all the granules around the vacuolar region indicates that the gel is again homogeneous.

The presence of a viscous mesh, interstices of which are filled with the fluid phase, is thus in accord with the observed vacuolar cycle and is an hypothesis which adequately accounts for differences between the observed movements of the vacuome granules and the osmiophilic granules. Mast (1926) describes small fluid vacuoles in the gelated ectoplasm of *Amoeba*. The reticulum described above is similar to Mast's concept with the exception that the fluid phase is continuous rather than in discrete vacuoles as in *Amoeba*.

IDENTIFICATION OF GOLGI MATERIAL IN CILIATES

Various criteria have been suggested by different authors to facilitate the identification of Golgi material. Hirschler (1927) stressed the importance of positive identification of mitochondria in order to eliminate confusion of Golgi material with the chondriome. Bowen (1928*b*) suggested in addition that consistent impregnation by both the silver and osmic methods would be good proof. He also stressed resistance to bleaching in osmic impregnated material as a character of true Golgi material. Hall (1931) used as criteria the above staining reactions and in addition stressed the idea that the Golgi material is probably quite similar in morphology and distribution in all Protozoa. He therefore restricts the term Golgi material to those inclusions which can be demonstrated consistently by the standard methods and which are similar in form and intracytoplasmic distribution in all Protozoa.

The morphology of the osmiophilic materials in ciliates is still a matter of dispute. The great majority of workers in this field describe small granules as Golgi material, but a few, led by Nassonow (1924), homologize either the wall of the contractile vacuole or a shell around the wall of the contractile vacuole (Strelkow, 1931*b*) with Golgi material.

The workers demonstrating a solid membrane have used the warm impregnation method advocated by Nassonow (1924). Hirschler (1924) showed that this warm method tends to produce over-impregnation, and in closely packed spherules the osmic precipitate spreads and obscures the true granular nature of the region. He advocates a cold method of impregnation to eliminate this source of error. Impregnation of the Ophryoscolecidae strikingly bears out this view. The heavy black membrane around the vacuole visible in material treated by the warm impregnation method (pl. 17, fig. 26) is shown by cold impregnation to be granular (pl. 17, fig. 25) and *this granular composition of the vacuolar region is seen in living material.* The figures showing solid impregnation of the vacuolar walls of *Chilodon* and *Dogielella,* published by Nassonow (1925), of *Balantidium* by Bojewa-Petruschewskaja (1928), and of the Cycloposthiidae by Strelkow (1931*b*) indicate a marked granular roughening on the outer margin of the osmiophilic layer, giving evidence that these solid black membranes are the result of over-impregnation of an originally granular zone. The blackened membranes or walls of vacuoles are therefore interpreted as being heavy aggregations of osmiophilic granules rather than solid structures. The variations in the impregnation of regions around the contractile vacuoles in different ciliates may then be explained as representing a difference in the localization of the formation of the contractile vacuole. A series extending from practically no aggregation of osmiophilic granules to a very heavy aggregation around the vacuoles has been demonstrated in the Ophryoscolecidae and has been shown to be correlated with the degree of localization of the formation of the contractile vacuoles. The main difference between such forms as *Spirostomum* (Hirschler, 1924*a*) and *Metadinium* is that in the former genus the osmiophilic granules are scattered more or less uniformly throughout the cytoplasm, while in the latter many are concentrated around each vacuole.

Certain granules have been described, however, which are obviously not to be considered as homologous with the granules discussed above. Richardson and Horning (1931) showed that the pyriform granules described by King and Gatenby (1926) in *Opalina* are not Golgi granules. The macronuclear knobs of *Stentor* described by Park (1929) also must be excluded.

The criterion of constancy of morphology and distribution in all Protozoa is a logical one and appears to be fairly valuable unless it is construed so strictly as to rule out any possibility of a cyclical change in morphology or in distribution of the granules. Hall applies this criterion very strictly, sometimes selecting the true Golgi material from other blackened material merely because of greater frequency of occurrence. Such a demand that Golgi material be absolutely constant in distribution and morphology would rule out the Golgi material of gland cells which show marked cyclic changes (Bowen, 1928*b*). Even allowing for cyclic changes, the criterion of constancy of form is valuable only in a negative way. The Golgi material usually accepted as such in the Protozoa is granular in form. This helps to rule out membranes, macronuclear knobs, or parabasal bodies of flagellates, but does not help to distinguish true Golgi granules from other cytoplasmic granules.

Granules which satisfy the criteria of staining reactions but which differ in other properties have been demonstrated in various ciliates. Hall (1931) showed that the granules vitally stained with neutral red may be subsequently impregnated by exposure to osmium tetroxide solution for from 3 to 4 days. Peschkowskaja (1928) showed that the osmiophilic granules of *Climacostomum* could be demonstrated vitally with neutral red, but also showed that neutral red reveals at the same time many granules which are not osmiophilic. In the Ophryoscolecidae, the osmiophilic granules and the granules demonstrable with neutral red are two entirely separate sets of cytoplasmic inclusions. A puzzling phenomenon found in the Ophryoscolecidae may be included here. The only granules in *Eudiplodinium* which can be impregnated with osmic acid are those found in the vacuolar region. It was shown from a study of living material that this region is composed of granules from the surrounding ectoplasm. Therefore these granules, as they assemble in the vacuolar region, undergo some chemical change or, more likely, some physical change which permits of their impregnation by the osmic technique. It is evident that, in ciliates, Golgi material identified by means of staining reactions includes many granules similar in a general way but differing in various details of their staining reactions or in solubility.

It is somewhat questionable then to homologize granules on the basis of their reaction to osmic or silver techniques and it is difficult to homologize them with the Golgi material in the Metazoa. *On the basis of the criteria now available,* however, the osmiophilic granules described in the Ophryoscolecidae are considered to be homologous with the osmiophilic granules described as Golgi material in various other ciliates. They are small granules which can be selectively impregnated by means of the silver or osmic techniques and can be separated from the other cytoplasmic granules, such as mitochondria and glycogen, by appropriate staining methods. The granules are usually found throughout the ectoplasm. They also show a cyclic aggregation around the contractile vacuoles, which is comparable to the changes occurring in the Golgi material in gland cells. Since they do not stain with neutral red, they are not strictly comparable to the vacuome granules described by Hall (1931) in *Stylonychia* and by Dunihue (1931) in *Paramecium.*

The vacuolar region described in the Ophryoscolecidae is strikingly similar to the Golgi region of secretory cells. Both regions are specialized parts which show marked cyclical changes in connection with the expulsion of material from the cell. The cytoplasmic components found in the Ophryoscolecidae and their observed activity correspond more nearly to the views on the activity of Golgi material expressed by Nassonow (1926) and by Bowen (1928*a*) than to the views of Parat (1928). There are three definite types of cytoplasmic components in these ciliates, the Golgi granules, the vacuome, and the chondriome. The Golgi granules can be identified in several of these ciliates when alive, because of the concentration of these granules in the vacuolar region. The findings agree thus far with those of Nassonow and Bowen. The Golgi granules contribute directly to the formation of the accessory vacuoles while neither the vacuome nor the chondriome in these ciliates shows any relation to the vacuolar cycle. The vacuolar region in these ciliates therefore does not conform in this respect with either Bowen's or Parat's interpretation of the function of the Golgi material.

The vacuolar region found in these ciliates shows definite evidence of the elimination of materials by means of the vacuolar fluid and corresponds to the secretory region or "region of Golgi" in gland cells. The nature of the materials eliminated by the vacuolar region was not determined in this investigation. Since, however, the pellicle in the Ophryoscolecidae has been shown to be relatively impermeable and since the vacuolar system is the only demonstrable path by which materials are constantly being passed to the exterior, it is likely that the katabolic wastes of these ciliates are eliminated by this organelle rather than by direct diffusion through the pellicle.

SUMMARY

1. The cycle of the contractile vacuoles was studied in both living and fixed material in the following genera of the Ophryoscolecidae: *Ophryoscolex, Epidinium, Ostracodinium, Polyplastron, Eudiplodinium,* and *Metadinium.*

2. The ectoplasm of these forms is very granular with no alveoli or vacuoles except those found in connection with the contractile vacuoles.

3. There is a permanent excretory pore for each vacuole in this group of ciliates. It opens into a canal formed by the pellicle. The inner end of the canal is closed by a thin membrane except during systole. The wall of the vacuole is a temporary structure. It is never impregnated by either the osmic or the silver techniques.

4. The contractile vacuole in all genera is formed by the coalescence of small accessory vacuoles. The accessory vacuoles arise *de novo* in small solated portions of the ectoplasm. The contractile vacuoles in *Ophryoscolex* and *Epidinium* arise in the whole ectoplasm while those of *Ostracodinium* are restricted in origin to a narrow dorsal strip of the ectoplasm. The accessory vacuoles of *Eudiplodinium* and *Metadinium* are formed only in a sharply defined granular region around the contractile vacuole.

5. The vacuolar fluid is mainly derived from excess fluid taken in by the cystostome during feeding. The general pellicular covering of the body is relatively impermeable and admits little if any fluid. Excretion by diffusion through the pellicle is unlikely.

6. The cycle of the contractile vacuole in all these genera is in three parts: the resting period, systole, and diastole. The resting period is marked by gelation of the surrounding ectoplasm and is a completely static stage. Systole is initiated by a solation of the surrounding ectoplasm and rupture of the membrane over the pore. Systole may be complete or only partly complete. Contact of the contractile vacuole with the excretory pore does not initiate systole. The closure membrane is re-formed from a remnant of the wall of the old contractile vacuole. The early part of diastole is marked by the formation and fusion of accessory vacuoles to form the new contractile vacuole which then becomes attached to the excretory pore. The surrounding cytoplasm then reverts to the gel state and a period of growth takes place. When this ceases, the resting stage commences.

7. Mitochondria, glycogen granules, granules stainable with neutral red, and osmiophilic granules were identified. The osmiophilic granules and the granules stainable with neutral red are interpreted as being the Golgi material and the vacuome, respectively.

8. Osmiophilic granules accumulate around the contractile vacuole during the early part of diastole and then are gradually reduced in number. This

granular vacuolar region can be noted in living material. The formation of accessory vacuoles in these granular regions involves a solution of these granules in the vacuolar fluid. This represents a possible method for the elimination of katabolic wastes.

9. Neither the vacuome nor the chondriome show any relation to the pulsatory cycle.

10. The ectoplasm around the vacuolar region during systole shows evidence of the presence of a coarse, viscous reticulum in which are embedded the granules of the vacuome and with the interstices filled with fluid ectoplasm in which the osmiophilic granules lie. The osmiophilic granules are thus free to move into the vacuolar region, while the viscous reticulum retains the vacuome granules.

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EXPLANATION OF PLATES

All whole mounts and sections, unless otherwise noted, were fixed in Champy's fluid and then impregnated with osmium tetroxide at from 20° to 24° C for a period of from 16 to 21 days, then bleached 48 hours in turpentine. The sections were cut 5μ thick. All drawings were made with the aid of a camera lucida.

PLATE 15

Photomicrographs by Mr. J. E. Gullberg.

Fig. 1. *Epidinium caudatum,* × 550. Dorsal view of the posterior vacuole in diastole.

Fig. 2. *Ophryoscolex caudatus,* × 900. Cross-section at the level of the posterior circlet of vacuoles. Da Fano silver impregnation.

Fig. 3. *Metadinium medium,* × 650. Parasagittal section of the anterior vacuole. This vacuole is near the end of diastole. Compare with pl. 16, fig. 31.

Fig. 4. *Epidinium caudatum,* × 550. Right lateral view of whole mount. An over-impregnated specimen showing complete blackening of the vacuoles.

Fig. 5. *Ostracodinium monolobum,* × 550. Left lateral view of whole mount. All five vacuoles are in the resting stage and are surrounded by minute accessory vacuoles. Compare with pl. 17, fig. 42.

Fig. 6. *Ophryoscolex caudatus,* × 550. Frontal section showing a pair of vacuoles in diastole.

Fig. 7. *Polyplastron multivesiculatum,* × 550. Cross-section at the level of the anterior ventral vacuole.

Fig. 8. *Eudiplodinium maggii,* × 650. Right lateral view of whole mount. Both vacuoles are in the early stage of diastole.

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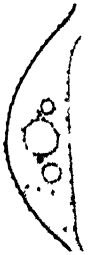
PLATE 16

Figs. 9–15. *Ophryoscolex caudatus,* × 1500. Fig. 9, beginning of systole, cross-section; fig. 10, diastole, longitudinal section; fig. 11, diastole, longitudinal section; fig. 12, diastole, cross-section. Da Fano silver impregnation; fig. 13, diastole, cross section; fig. 14, diastole, cross-section. The large glycogen granules with dark centers are shown in addition to the osmiophilic granules. Osmic impregnation, stained with Sudan III; fig. 15, resting stage, longitudinal section.

Figs. 16–19 *Epidinium caudatum,* × 1500, whole mounts. The posterior vacuole in dorsal view showing in addition to the vacuole the permanent excretory pore and pellicular striations. Fig. 16, diastole; fig. 17, late diastole; figs. 18–19, resting stages.



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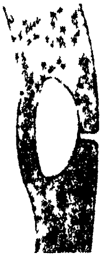

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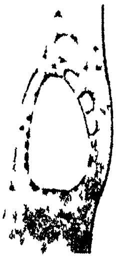

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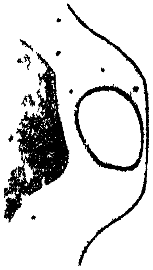

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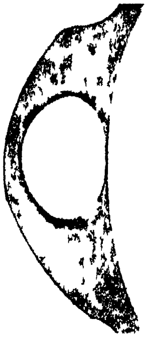

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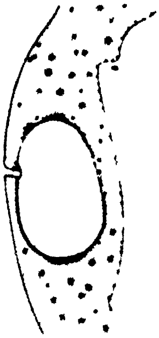

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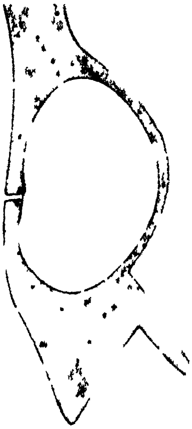

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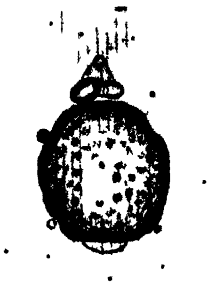

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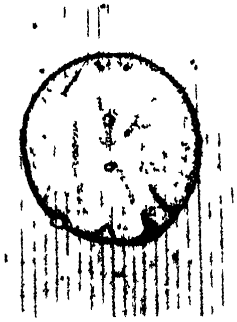

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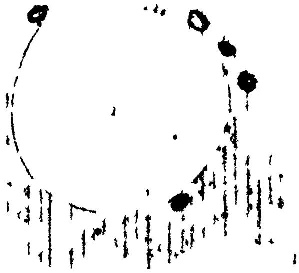

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PLATE 17

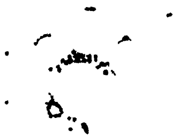
Figs. 20–28, 33. *Polyplastron multivesiculatum*, × 1650. Cycle shown by the dorsal vacuoles. Fig. 20, early diastole, frontal section; fig. 21, diastole, sagittal section; fig. 22, diastole, frontal section; figs. 23–24, middle of diastole, longitudinal sections; figs. 25–26, diastole, a comparison of the results of impregnation at 20°–24° C (fig. 25) and impregnation at 30°–32° C (fig. 26), frontal sections; fig. 27, late diastole, cross-section; fig. 28, late diastole, cross-section; fig. 29, resting stage, sagittal section; fig. 33, late diastole, sagittal section.

Figs. 30–32. *Metadinium medium*, × 800. Fig. 30, early diastole, cross-section; fig. 31, late diastole, parasagittal section; fig. 32, late diastole, cross-section. Photomicrographs by Mr. J. E. Gullberg.

Fig. 34. *Eudiplodinium maggii*, × 700. Posterior vacuole in early diastole. Frontal section. Compare with pl. 17, fig. 44.

Fig. 35. *Ostracodinium monolobum*, × 800. Anterior vacuoles in diastole, frontal section.

Fig. 36. *Eudiplodinium maggii*, × 250. Anterior vacuole in the resting stage, the posterior vacuole in early diastole, whole mount. Compare with pl. 17, fig. 47.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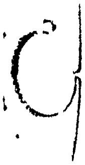
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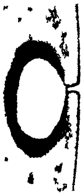
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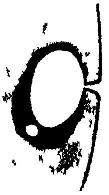
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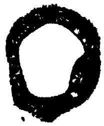
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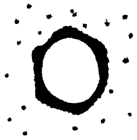
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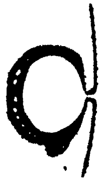
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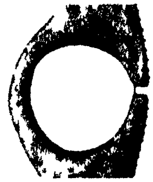
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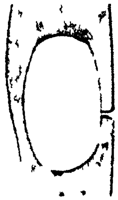
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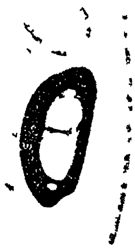
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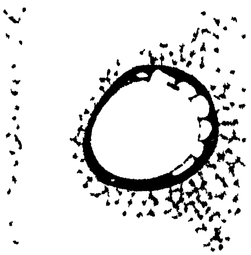
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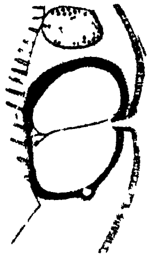
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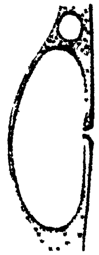
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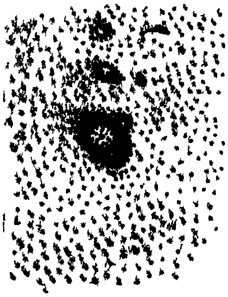
PLATE 18

Figs. 37–38, 40–43. ***Ostracodinium monolobum***, **× 660. Fig. 37, early diastole, frontal section; fig. 38, diastole, frontal section; fig. 40, late diastole, whole mount; fig. 41, diastole, sagittal section; fig. 42, resting stage, whole mount; fig. 43, late diastole, frontal section.**

Fig. 39. ***Epidinium caudatum***, **× 1500. Anterior vacuole in resting stage, cross-section.**

Figs. 44–49. ***Eudiplodinium maggii***, **× 1500. Fig. 44, posterior vacuole in diastole, frontal section; fig. 45, late diastole, frontal section; fig. 46, resting stage, frontal section; fig. 47, posterior vacuole at the beginning of systole; fig. 48, diastole, whole mount; fig. 49, late diastole, whole mount.**



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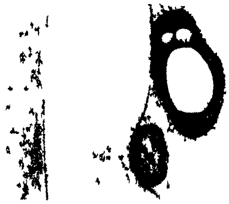

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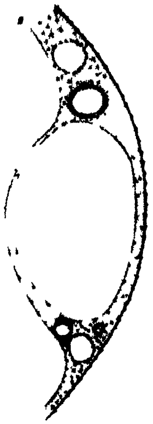

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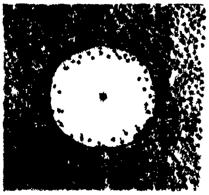

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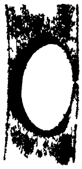

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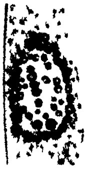

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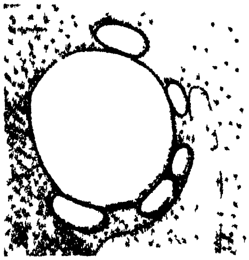

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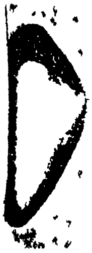

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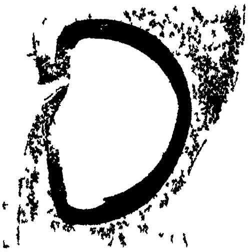

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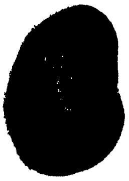

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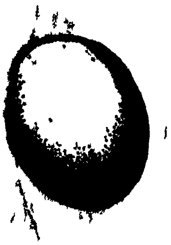

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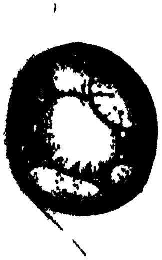

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THE PULSATORY CYCLE OF THE CONTRACTILE VACUOLES IN THE OPHRYOSCOLECIDAE, CILIATES FROM THE STOMACH OF CATTLE

BY

RONALD F. MacLENNAN

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THE PULSATORY CYCLE OF THE CONTRACTILE VACUOLES IN THE OPHRYOSCOLECIDAE, CILIATES FROM THE STOMACH OF CATTLE

BY
RONALD F. MACLENNAN

INTRODUCTION

The contractile vacuoles of the Protozoa are both obvious and active organelles and have long been the objects of considerable study. In spite of this, the contractile vacuoles of the Ophryoscolecidae have attracted far less attention than any other of the organelles of this large group of ciliates. The vacuoles of the Ophryoscolecidae are large, have a relatively slow pulsatory cycle, are fixed in location, and are covered with a relatively smooth and transparent pellicle. They thus afford highly favorable material for observations on both living and fixed material and also give an opportunity to compare conditions in a number of highly diversified but closely related genera.

The purpose of this paper is to give an account of the pulsatory cycle of the contractile vacuoles in the Ophryoscolecidae with particular emphasis on the following points which are of interest in connection with the problem of contractile vacuoles in general: (1) formation, growth, and discharge of the vacuoles; (2) the sol-gel phenomena exhibited by the cytoplasm during the pulsatory cycle; (3) the excretory pore and the mechanism of its action; (4) the relationship between the vacuole and the various components of the cytoplasm.

I wish to express my appreciation of the very helpful advice and criticism given to me by Professor C. A. Kofoid and Professor Harold Kirby, Jr., during the course of this investigation.

MATERIAL AND METHODS

The Ophryoscolecidae, together with numerous holotrich ciliates, are found in the first, second, and third stomachs of ruminants. They are found in greatest abundance and in best condition in the second stomach (reticulum). The ciliates used in this investigation include one or more species of each of the following genera: *Epidinium, Eudiplodinium, Metadinium, Ophryoscolex, Ostracodinium,* and *Polyplastron.* All genera except *Ophryoscolex* were found in both cattle and sheep; *Ophryoscolex* was found only in sheep.

The material from the second stomach was obtained from cattle and sheep slaughtered in Oakland, California. The contents were removed on the killing floor and brought to the laboratory in tightly corked Dewar flasks. The ciliates remain alive twenty-four hours or longer in material kept in this manner. Preparations sealed with vaseline were studied under a microscope fitted with a warm stage. The activity of the ciliates was controlled by adjusting the temperature of the warm stage.

Comparison of fresh material and material from ten to twelve hours old showed that the ciliates kept in a Dewar flask for more than twelve hours exhibit the beginnings of degeneration of the cytoplasm, the most obvious mark being a general, irregular vacuolization of the ectoplasm. In order to eliminate any artifacts from this source, no material was studied later than six hours after taking it from the host, and no slides of live ciliates were studied later than one hour after they were prepared. In addition, only those ciliates which showed normal action of the membranelle zones were used.

Standard staining methods were followed as closely as possible in order to give results which might be compared with those of the majority of other workers.

Janus green B, Bismarck brown, neutral red, and neutral violet were used to demonstrate mitochrondria and the vacuome in live material by the film method described by Hall (1929). These stains used in one-tenth to one-half per cent solution in the stomach contents are specific for the cytoplasmic granules and are not toxic to the ciliates.

In order to test the reaction to osmic vapor of the granules staining with neutral red ("vacuome" according to Hall, 1931), coverslip preparations of live ciliates were inverted over a depression slide containing 2 per cent osmium tetroxide solution and sealed in place with vaseline. Camera lucida sketches of the arrangement of granules in a restricted region of cytoplasm were made before the neutral red faded out. At frequent intervals over a period of seven days the preparations were compared with the original sketches. The granules stained with neutral red were never selectively impregnated by the osmic treatment, but were darkened along with the general cytoplasm. Occasionally granules which had not been stained with neutral red showed a slight darkening after two or three days. We conclude that these granules which may be stained with neutral red are not osmiophilic, and are not identical with the osmiophilic granules.

Both osmic and silver impregnations were made according to the methods recommended by Bowen (1928*a*) for the demonstration of Golgi material. The silver impregnations were made at room temperature (21° C.), while the osmic impregnations were made both at room temperature and at 32° C. The cold method is far more satisfactory than the warm method. Successful impregnations showed blackened granules in 95 per cent of the ciliates. Some of the ciliates impregnated in osmium tetroxide were stained by Altmann's aniline-fuchsin-picric acid method in order to demonstrate mitochondria along with the osmiophilic granules.

The different species were segregated by means of a fine pipette, imbedded in paraffin stained with Sudan III, and sectioned at two, five, or seven microns.

Small granules in the ectoplasm of all the species investigated and the small prisms of the skeletal plates were found to be stained with Sudan III. Granules similar in size and distribution were stained brown by chlor-zinc-iodide, which is used as a test for glycogen. Several sections stained with Sudan III were drawn with the aid of a camera lucida and then restained with chlor-zinc-iodide. Comparison with the camera lucida sketches showed that the red granules and prisms are stained brown. It seems, therefore, that the above method of using Sudan III reveals glycogen granules.

GENERAL DESCRIPTION OF THE CYTOPLASM

The boundary layer, a thin, hyaline membrane, divides the cytoplasm of the Ophryoscolecidae into ectoplasm and endoplasm. This layer, which is 0.2μ or less in thickness, appears homogeneous in both living and fixed material. A fibrillar structure in the membrane has been described by Eberlein (1895) and more recently by Rees (1931). Sharp (1914) pointed out that the presence of fibrils pressed close to the boundary layer probably led to this interpretation. This criticism is particularly applicable to the genus *Metadinium* which Rees investigated (his *Diplodinium medium*). All our observations bear out the homogeneity of the boundary layer. The cytoplasm is composed of a clear matrix in which are imbedded innumerable spherical granules, from 0.2 to 0.5μ in diameter.

The only movement present in the ectoplasm is Brownian movement of the granules and weak localized currents in connection with the contraction of the vacuoles. In the endoplasm, in addition to pronounced Brownian movement of the granules there is cyclosis. Both parts of the cytoplasm are in the sol state, with the endoplasm much less viscous than the ectoplasm. Localized areas of gelation, which occur at times in the ectoplasm near the contractile vacuoles, will be described in detail in the parts dealing with the vacuolar cycles. No gelation was observed in the endoplasm of normal individuals.

Cytoplasmic granules may be either in direct contact with the matrix, or separated from it by a watery fluid. It was asserted by Taylor (1923) that the granules of *Euplotes patella* lie in a clear, watery vacuole surrounded by an envelope of relatively viscous cytoplasm. In some specimens, he could see this minute vacuole, in others he inferred it from the evident restriction of the Brownian movement of the granules. Mast (1926) reported a similar condition in the gelated portions of *Amoeba proteus*. He based his conclusions on observations of the narrow amplitude of the Brownian movement and on the retention by the granules of a fixed position in relation to each other. The Brownian movement of granules in the ciliates from cattle was studied carefully on this point. No watery vacuoles could be discerned around the granules, and they were continually changing position and tumbling around one another. In the gelated regions, no Brownian movement could be observed. Therefore the granules lie in direct contact with the clear, homogeneous matrix of the cytoplasm and not in small, watery vacuoles.

IDENTIFICATION OF CYTOPLASMIC GRANULES

The granules of the ectoplasm were studied carefully in order to identify those showing relationship to the contractile vacuoles. The only criteria for distinguishing different types of granules in the Ophryoscolecidae are the staining reactions, since the granules are usually indistinguishable in form or size.

Mitochondria.—Mitochondria were identified in living material by staining with Janus green B, and in fixed material by the Champy-Kull method or by staining with acid fuchsin and picric acid after osmic impregnation. The mitochondria are exceedingly numerous small granules uniformly distributed through the ectoplasm. They usually appear to be the smallest of the ectoplasmic granules, their order of magnitude being about 0.2μ.

Glycogen.—Glycogen granules occur abundantly in well fed Ophryoscolecidae. Iodine reagents (sulphuric acid followed by iodine; chlor-zinc-iodide; Lugol's solution) are useful for demonstrating glycogen granules temporarily, but are unsuitable for fine cytological preparations. Sudan III, used in the manner described above, does not interfere with the study of any cytological details and is therefore most useful in attempts to correlate the distribution of glycogen granules with that of other cytoplasmic inclusions.

The scattered glycogen granules are usually in the form of small spheres about 0.5μ in diameter. In chlor-zinc-iodide preparations they appear as homogeneous dark brown bodies with a rather irregular surface, but in sections stained with Sudan III, each granule appears as a smooth pink sphere with a brilliant red granule in the center. Occasionally the glycogen granules of *Ophryoscolex* appear as stout rods up to 1μ in length.

Glycogen is particularly abundant in the skeletal plates (fig. A, *1*), in the postero-ventral region of the body, and in the operculum. Since vacuome, chondriome, or Golgi material is often described in close connection with such reserves of food, a similar relationship was looked for in these ciliates. The glycogen granules, however, showed no close spatial relationship to the other cell inclusions (pl. 15, fig. 14).

Vacuome.—Neutral red stains specifically small spherical inclusions in many types of animal cells. The sum total of these inclusions has recently come to be known as the vacuome (Parat, 1928). Neutral red used on living Ophryoscolecidae consistently stains numerous granules ranging in size from

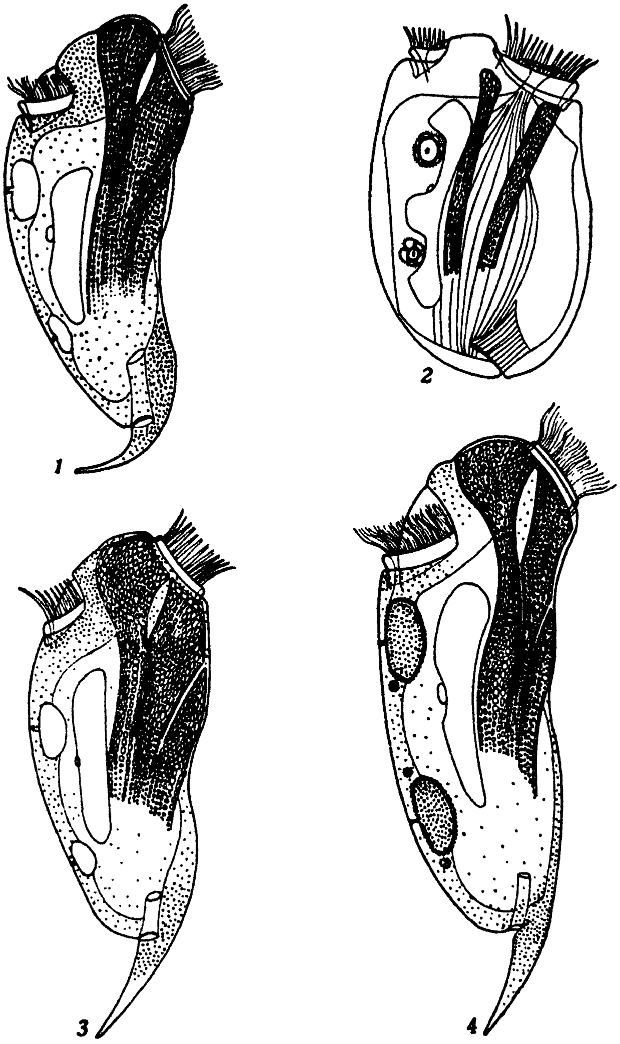

Fig. A. *1, 3, 4, Epidinium caudatum,* × 500, *1,* stained with chlor-zinc-iodide to show distribution of glycogen; *3,* stained vitally with neutral red to show distribution of vacuome; *4,* impregnated with osmium tetroxide to show distribution of osmiophilic granules; semidiagrammatic.

2, Metadinium medium, × 250, unstained live specimen, semidiagrammatic.

0.2 to 0.5μ. They are particularly numerous in the ectoplasm which forms the operculum, in that of the postero-ventral region, and in a narrow region just posterior to the membranelle zones (fig. A, *3*). These regions appear as solid red bands under low magnification, but under high magnification it is clearly seen that this appearance is caused by the staining of many of the granules, some of the granules and the cytoplasmic matrix remaining colorless. A small number of granules which stain with neutral red are scattered uniformly through the rest of the ectoplasm. A few of them usually adhere to the outer surface of the vacuolar membranes, but there is very definitely no concentration of the granules near these structures. The granules described above correspond in position, size, and reaction to neutral red to the vacuome described by Joyet-Lavergne (1926) and Hall (1931) in various Protozoa.

Osmiophilic granules.—Osmic and silver impregnation of the Ophryoscolecidae reveals a large number of granules specifically blackened. These granules are all less than 0.5μ in diameter, the largest ones occurring in *Eudiplodinium,* the smallest in *Polyplastron.* The granules are distributed generally throughout the ectoplasm (except perhaps in *Eudiplodinium*) and at certain times are very heavily concentrated around the contractile vacuoles (fig. A, *4*), sometimes giving the appearance of a heavy, thick, osmiophilic wall around the vacuole.

In the Ophryoscolecidae, the granules stained with neutral red are not blackened by osmic treatment, as pointed out in the discussion on technique; also the neutral red granules (fig. A, *3*) are never concentrated around the vacuoles as are the osmiophilic granules (fig. A, *4*). These observations show unmistakably that, in the Ophryoscolecidae, the neutral red granules and the granules demonstrated by osmic or silver techniques for Golgi materials are not identical; therefore the findings reported by Joyet-Lavergne (1926) and by Hall (1929–1931) are not applicable to all Protozoa.

The question of homologizing these osmiophilic granules with Golgi materials described in other Protozoa and in the Metazoa will be treated fully in the latter part of this paper. For the present, the noncommittal descriptive phrase "osmiophilic granules" will be used to designate them.

OBSERVATIONS ON THE VACUOLAR CYCLE

The vacuolar cycles found in *Epidinium* and *Eudiplodinium* are described in detail, since they show two widely differing types of contractile vacuoles. The cycles found in *Ophryoscolex, Ostracodinium, Polyplastron,* and *Metadinium* are described chiefly in respect to the differences brought out by comparison with *Epidinium* and *Eudiplodinium.*

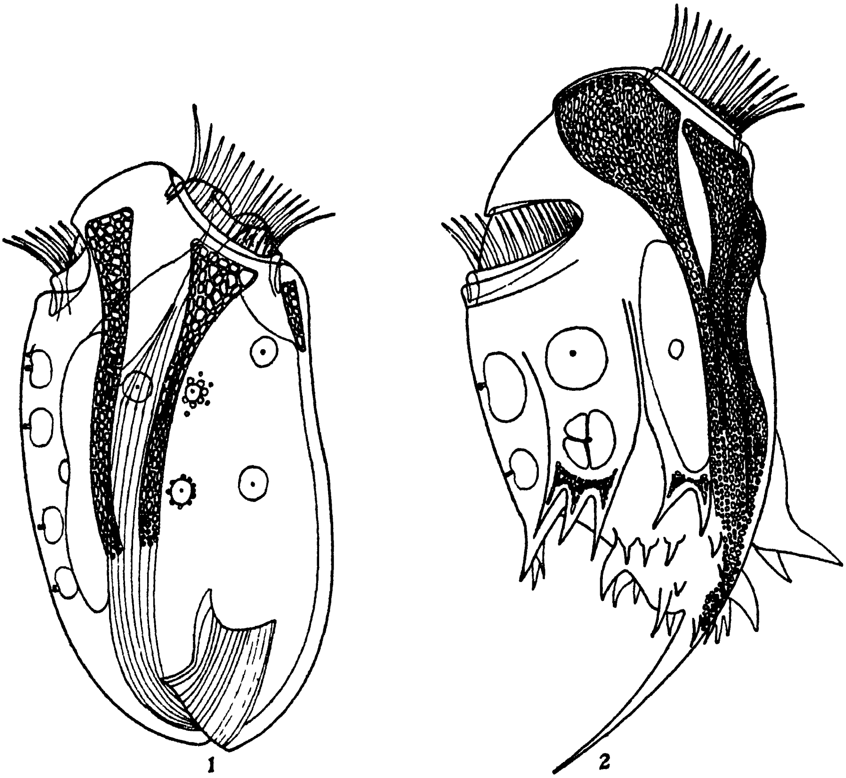

Fig. B. *1, Polyplastron multivesiculatum,* × 500; *2, Ophryoscolex caudatus,* × 500, semidiagrammatic.

CONTRACTILE VACUOLES OF EPIDINIUM

Epidinium caudatum (Fiorentini, 1889) was the commonest species in the material studied. *Epidinium ecaudatum* (Fiorentini, 1889), *E. bicaudatum* (Sharp, 1914), and *E. tricaudatum* (Sharp, 1914) were less common, but sufficient material was available to determine that the vacuolar cycle in these species is identical with that in *E. caudatum.*

Two vacuoles are present in *Epidinium* (fig. A, *3*). They lie along the dorsal mid-line, the anterior vacuole lying just behind the dorsal membranelle zone and the posterior vacuole near the level of the posterior end of the macronucleus. The anterior vacuole is slightly to the right of the posterior vacuole, due to the twisting of the body (Sharp, 1914).

The pore of each vacuole (fig. C, *7*) is a circular opening in the pellicle, from 1 to 2μ in diameter. From it a short tube, the excretory canal, extends inward for a distance of from 2 to 4μ. The wall of the canal is formed by a continuation of the pellicle which covers the body of the ciliate. A thin membrane is stretched over the inner end of the excretory canal, closing it during diastole.

The fully expanded vacuole (fig. C, *1*, *7*) is a simple lentoid vesicle flattened dorso-ventrally. The inner surface of the vacuole is convex, while the outer surface is slightly concave where the vacuole is pressed against the stiff excretory canal. In ten specimens the antero-posterior diameter ranged from 12 to 22μ, the dorso-ventral diameter from 8 to 13μ. The anterior vacuole in most specimens is from 2 to 4μ larger in both dimensions than the posterior vacuole. There are also many small accessory vacuoles around the main vacuole at this time. The vacuole in this stage may remain unchanged for a considerable period, which may be referred to as the resting stage.

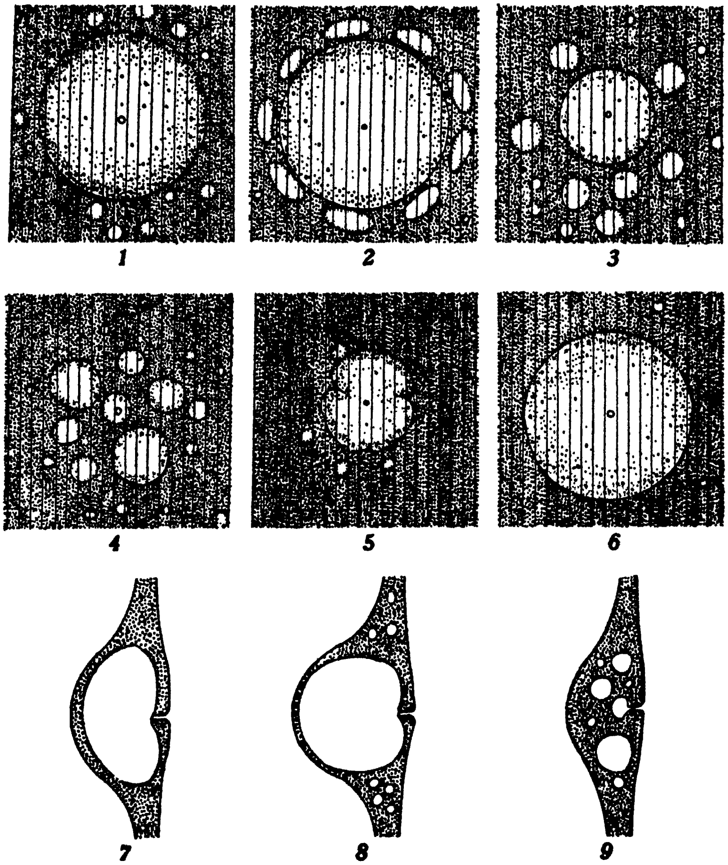

Fig. C. *Epidinium caudatum.* Contractile vacuoles during the pulsatory cycle drawn from live specimens stained vitally with neutral red. Granules stained vitally with neutral red are arbitrarily drawn larger than the non-staining granules. Semidiagrammatic, × 1000. *1–6*, dorsal view of anterior contractile vacuole; *7–9*, lateral view of anterior contractile vacuole in optical section. *1*, resting stage; *2*, late resting stage; *3*, systole; *4*, late systole; *5*, diastole; *6*, end of diastole; *7*, resting stage; *8*, vacuole rounded just before systole; *9*, systole.

Cycle.—The lentoid vesicle described above is surrounded by a region showing little, if any, Brownian movement, indicating a gel state. The first sign of activity is an increase of movement in the surrounding granules, accompanied by a rounding of the vacuole to a spherical shape (fig. C, *8*) slightly indented at one pole where it presses against the canal. This preliminary stage lasts about ten seconds. The vacuole then undergoes a sudden convulsion in adjusting itself completely on the canal. This is evidently the result of a further solation of the surrounding ectoplasm. The membrane sealing the pore ruptures at the same time and systole occurs immediately (fig. C, *4, 9*), lasting from 1.2 to 11.1 seconds and usually taking about 4 seconds. The excretory canal is again sealed by a membrane which is formed from the remnants of the wall of the old vacuole. Systole is usually complete, but from time to time incomplete systole in all gradations was observed. Several successive incomplete systoles were observed in three specimens. In these systoles the small remaining vacuole drew away from the canal and the wall of the vacuole was pulled out in a long string which finally broke. The part attached to the canal forms the closing membrane. The vacuole occasionally becomes spherical, the cytoplasm changing from gel to sol, without starting systole. The cytoplasm then gradually reverts to a gel state. If the sudden, final solation occurs, systole invariably follows.

The cytoplasm around the vacuole streams in during systole (fig. C, *3, 4*) carrying along the small accessory vacuoles. These vacuoles, along with any remnant of the old vacuole, then fuse to form the new vacuole (fig. C, *5*). This quickly comes in contact with the inner end of the canal, becomes attached to it, and remains there for the rest of the vacuolar cycle. The cytoplasm is in a sol state from the rounding up of the vacuole at the end of the resting stage to this early fusion of accessory vacuoles at the beginning of diastole. After this fusion of the accessory vacuoles the cytoplasm around the contractile vacuole reverts to a gel state which is marked by a slowing up and final cessation of Brownian movement and a marked irregularity in the outline of the vacuolar wall. This process of fusion continues for some time while the main vacuole grows to nearly full size. This early period of diastole occupies about one minute from the time of completion of systole, and in this time nearly all the accessory vacuoles become incorporated in the main vacuole (fig. C, *6*). The vacuole then increases in diameter without the addition of any more accessory vacuoles. The growth of the main vacuole gradually ceases and a new ring of accessory vacuoles appears around it. This ring corresponds to the V_2 group in *Euplotes* (Taylor, 1923) and is composed of vesicles which form the next contractile vacuole. The vacuole may remain quiescent in this state for some time, the usual period being about five minutes.

The time occupied by a single vacuolar cycle varied in the specimens observed by the writer from one minute to over an hour. The period of the early stage of diastole, the formation of the vacuole by fusion of the smaller vesicles, is constant, but the second stage, the growth without fusion of accessory vesicles, may be ended at any time by systole.

The small accessory vacuoles originate in all parts of the ectoplasm on the dorsal side of the ciliate. They first appear as minute vacuoles, about one micron in diameter, each with one or two colorless granules and occasionally one or two granules stainable with neutral red adhering to the surface. These small vacuoles migrate slowly toward the region of the main vacuole and at the same time fuse with the small vacuoles. No ectoplasmic currents could be observed which could account for this migration, the vacuoles appearing to force their way among the relatively motionless granules of the cytoplasm.

Relationship to osmiophilic granules.—The main vacuole in the quiescent phase of late diastole (pl. 16, fig. 19) appears in impregnated material as a clear vesicle limited by the yellowish orange wall and cytoplasm. A few blackened granules adhere to the vacuolar wall and others are scattered through the ectoplasm. The small accessory vacuoles surrounding the main vacuole are completely blackened by a swarm of osmiophilic granules. These granules are often so closely spaced that the accessory vacuoles appear to have homogeneous black walls with a rough, granular, outer surface.

No stages which could be interpreted as systole were found. Since systole is so short, this is rather to be expected.

The vacuole in early diastole (pl. 15, fig. 1; pl. 16, fig. 16), is a small vesicle with many black granules covering its surface and small black accessory vacuoles near it or in various stages of fusion with it. The number of osmiophilic granules adhering to the wall gradually diminishes in the larger vacuoles (pl. 16, fig. 17) until a stage is reached in which the vacuole is clear except for a few granules on its surface (pl. 16, fig. 18) and with very few accessory vacuoles grouped around it. This corresponds to the appearance of the living vacuole at the end of the first growth period. From then on, the main vacuole is surrounded by more and more small blackened vacuoles of the V_2 group. The blackened granules, as shown both in whole mounts and in sections (pl. 18, fig. 39) are always outside the vacuole, never inside. The membrane itself is not impregnated, the appearance of impregnation being caused by the close packing of granules on the surface.

The smallest accessory vacuoles observed had only one or two osmiophilic granules adhering to their surfaces. More granules are found around slightly larger vacuoles, until finally, in the accessory vacuoles near the main vesicle, it is difficult to see the clear vesicle in the middle of the aggregation of granules. This accumulation of osmiophilic granules around the accessory vacuoles and around the main vacuole during diastole indicates that these granules are probably closely connected with the concentration of materials within the vacuole.

CONTRACTILE VACUOLES OF EUDIPLODINIUM MAGGII

Two large contractile vacuoles lie beneath the right dorsal surface of *Eudiplodinium maggii* (pl. 15, fig. 8). The outer exretory pore of each vacuole is an ellipsoid from 3 to 8μ long and from 2 to 5μ wide, with its long axis lying parallel to the main axis of the body. These pores open on the right dorsal surface instead of along the dorsal mid-line as in most of the other genera. The excretory canal (fig. D, *7–9*) extends inward from the pore for the considerable distance of from 10 to 15μ and tapers rapidly, so that the inner pore is only from 2 to 4μ in diameter. This canal is formed by an extension of the pel-

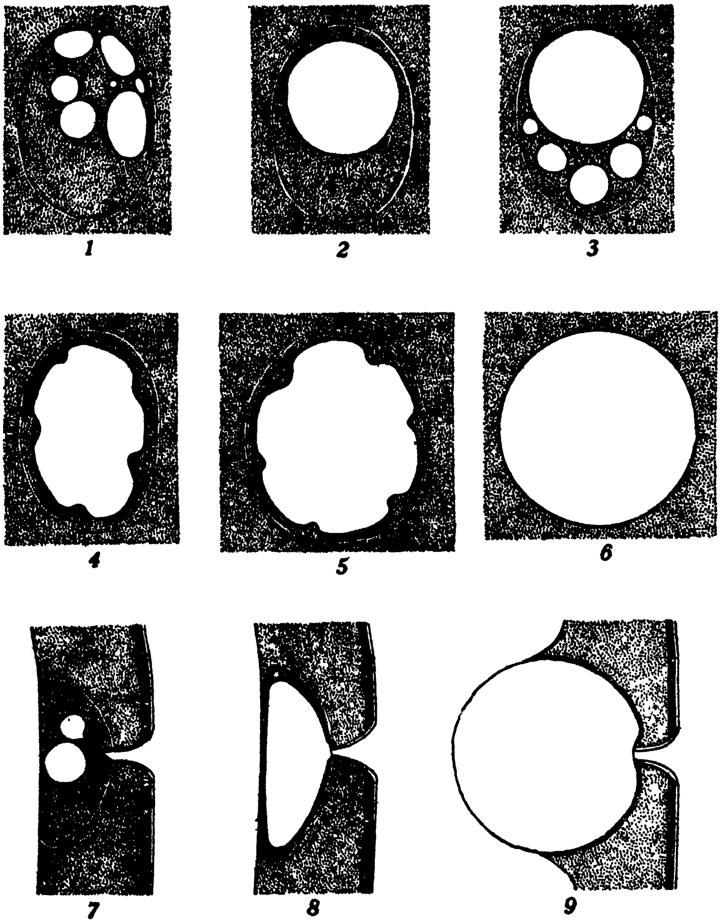

Fig. D. *Eudiplodinium maggii*, × 1000. Anterior contractile vacuole during the pulsatory cycle from a specimen stained vitally with neutral red. Granules staining with neutral red are arbitrarily drawn larger than the non-staining granules. Semidiagrammatic. *1–6*, lateral view in optical section; *7–9*, dorsal view in optical section. *1*, end of systole; *2–3*, first stage of diastole; *4–5*, second stage of diastole; *6*, vacuole rounded just before systole; *7*, end of systole; *8*, end of first stage of diastole; *9*, vacuole rounded just prior to systole.

licle, as in other genera. The pellicle covering the whole body has two distinct layers (pl. 18, figs. 44–46), an inner striated layer (from 1 to 2μ in thickness) and an outer clear layer (less than 0.5μ in thickness). Only the outer layer is continued inward to form the canal, which pierces the striated layer.

The resting vacuole is a large, clear vesicle (pl. 18, fig. 46), slightly elongated along the antero-posterior axis. The length of ten different resting vacuoles ranged from 20 to 30μ, the dorso-ventral diameter from 15 to 22μ. There is no specialized region of cytoplasm around the vacuole at this stage, nor are there any accessory vacuoles in the general ectoplasm. The granules near the vacuolar membrane show only a small amount of Brownian movement.

Cycle.—In preparation for systole the resting vacuole rounds up for a period of from 5 to 8 seconds (fig. D, *6, 9*), following a preliminary lowering of the viscosity of the cytoplasm immediately surrounding the vacuole. Then a further solation takes place, the vacuole completes the rounding up process in a sudden convulsion, and systole immediately follows. Systole takes from 2.1 to 10 seconds and is usually complete, the remnants of the vacuolar wall forming the membrane closing the inner excretory pore. In some specimens systole was arrested after varying amounts of fluid had been expelled. When only a small vacuole was left (fig. D, *7*) this was pulled away from the pore, apparently by the strong currents in the ectoplasm of the vacuolar region. The vacuolar wall was pulled out in a narrow thread which finally broke, one end was resorbed into the wall of the freed vacuole, the other end formed a smooth membrane across the inner pore. In a very short systole only a small amount of fluid is expelled, and the closing membrane is apparently formed by a slight constriction of the vacuolar wall just below the inner pore.

The ectoplasmic matrix carrying with it a host of small non-stainable granules rushes in during systole (fig. D, *1*) behind the retreating wall of the vacuole. The granules stainable with neutral red remain stationary. The general ectoplasmic mass around the vacuole fills in a part of the space formerly occupied by the resting vacuole. Thus at the end of systole there is a definite region beneath the excretory canal somewhat smaller than the resting vacuole and containing a large number of granules which do not stain with neutral red, in violent Brownian movement and also tumbling around as if there were strong but exceedingly localized currents in the clear matrix. The general ectoplasm containing both clear non-staining granules and granules stained with neutral red surrounds this region, and the granules in this region show very little Brownian movement as compared to their movement in the vacuolar region. A very narrow, almost invisible, hyaline layer separates the two regions.

Accessory vacuoles arise in this specialized region even while it is forming and before the old vacuole is completely discharged. These vacuoles tend to arise along the anterior border and often outline it clearly. Accessory vacuoles forming in the middle float around freely and often touch, but show no tendency to fuse until after systole is complete. They then fuse (fig. D, *2*) forming a single, large vacuole almost completely filling the anterior portion of the vacuolar region. More vacuoles form in the remainder of the granular area (fig. D, *3*) and fuse with the first vacuole. This formation of accessory vacuoles and subsequent fusion ceases when only a narrow band of granular material remains around the clear vacuole (fig. D, *4, 8*) and brings to an end the first growth period in from thirty seconds to two minutes after the completion of systole.

Extreme solation of the material within this vacuolar region marked it at the beginning and this continues until after the accessory vacuoles begin to fuse. The Brownian movement and turbulence of the ectoplasm of the vacuolar region then gradually diminishes until at the end of the first growth period the wall of the vacuole is sufficiently gelated to retain traces of the accessory vacuoles in the roughened outline of its walls (fig. D, *4*). Outside of this region, however, the consistency of the cytoplasm, as indicated by Brownian movement, shows no such wide fluctuations.

This vacuole with roughened walls continues to enlarge in all directions but without the formation of any accessory vacuoles (fig. D, *5*). The granular zone gradually becomes thinner, and the outline of the vacuole becomes smoother until the elongate resting vacuole with no specialized granular region around it is formed anew. This second growth period lasts from one to two minutes. The resting stage lasts from fifteen seconds to an indefinite length of time.

The formation of the accessory vacuoles in *Eudiplodinium maggii* is remarkable in that it is strictly limited to a short period during systole and to the first growth stage and also is limited in locality to this small, specialized vacuolar region. No accessory vacuoles are formed at any other time or place. Occasionally small vacuoles do appear in the surrounding cytoplasm in individuals from material kept too long, but no such vesicles formed outside of the vacuolar region were ever observed to contribute to the contractile vacuole in any way. One such vesicle lying adjacent to the anterior vacuole was observed to be unchanged throughout seven consecutive cycles of this vacuole.

The formation of an accessory vacuole is first marked by a gradual diminution of granules in a restricted region and increase of movement in the remaining granules. This lasts from five to ten seconds during which time the granules completely disappear. Then very suddenly a differentiation is observable between the clear central region and the surrounding granular region, and a small accessory vacuole, bounded by a distinct protoplasmic wall, becomes apparent.

Relationship to osmiophilic granules.—The resting vacuole in individuals impregnated with osmic acid is a large, clear vesicle surrounded by golden yellow cytoplasm (pl. 18, fig. 46). Few granules occur either in connection with the vacuole or in the general ectoplasm. The next stage found in fixed material was that just at the completion of systole (pl. 17, fig. 36; pl. 18, fig. 47). The vacuole in this stage is a small vesicle completely filled with thickly packed, heavily blackened granules. A clear central vacuole bounded by a gray reticulum then appears (pl. 15, fig. 8; pl. 17, fig. 34; pl. 18, figs. 44, 48), usually in the anterior portion of the granular mass, and in other later stages more clear vacuoles appear (pl. 18, fig. 49). This vacuolization of a dense, granular osmiophilic region corresponds exactly to the vacuolization of the granular vacuolar region observed in living material.

The absolutely sharp demarkation of locality of granules in *E. maggii* furnishes further evidence that the granules stainable with neutral red are entirely different from the osmiophilic granules and that the two types of granules perform two entirely different functions. The osmiophilic granules are found only within the vacuolar region, while the granules stainable with neutral red are concentrated outside of it and very few, if any, are found within the vacuolar region.

Kraschenninikow (1929) has previously described the results of osmic impregnation in *E. maggii.* He described both the black granules and the gray reticulum. He claims that the vacuolar membrane itself was impregnated, the granular appearance being caused by unevenness in the membrane. The black granular portion and the gray reticulum were homologized with the intensely osmiophilic "apparat externum" and the less intensely osmiophilic "apparat internum" respectively, described by Hirschler (1927) in the Golgi apparatus of various metazoan and protozoan cells. The presence of black granules and a gray reticulum was confirmed in the present investigation, but no evidence was found to support Kraschenninikow's statement that the osmiophilic granules are a part of the vacuolar membrane.

Origin of accessory vacuoles.—The formation of accessory vacuoles in *E. maggii* apparently occurs by the solution of the osmiophilic granules, first in localized regions and finally involving all of them. Sections of impregnated vacuoles in early stage of diastole with accessory vacuoles forming is very suggestive in regard to the rôle of the osmiophilic granules in the formation of accessory vacuoles. These granules are massed in a heavy band around a central cavity (pl. 18, fig. 44). Close examination shows that they are suspended in a fine reticulum, which was turned a dark gray by the action of the osmium tetroxide. This gray reticulum also fills the spaces between small clear vesicles within the granular band. Later stages show a large clear vesicle, evidently formed from a fusion of the smaller, clear vesicles (pl. 18, fig. 45) and by the reduction and finally the disappearance of the granular band.

The evidence for the solution of the osmiophilic granules is much more convincing if certain phenomena observed in living material are considered along with the evidence from fixed material. In systole the migration of a large number of granules into the vacuolar region is easily observed, but in diastole no movement outward could be observed, although the granules unmistakably disappear and are replaced by the clear, watery, vacuolar fluid. The direct observation of the solution of granules does not seem to be feasible owing to their large number and incessant activity which make it impossible to follow continuously any one granule or set of granules. The observation nearest to direct proof of solution is that, just prior to the formation of any particularly accessory vacuole, the number of granules in that region is greatly reduced and they disappear completely as the accessory vacuole is finally formed. Therefore, from a consideration of both living and fixed material, the conclusion that the granules dissolve in the vacuolar fluid seems unavoidable.

CONTRACTILE VACUOLES OF OPHRYOSCOLEX CAUDATUS

Ophryoscolex caudatus Eberlein 1895 (fig. B, *2*) possesses nine contractile vacuoles arranged in four longitudinal pairs around the middle portion of the body, with the ninth vacuole in the portion just ventral to the skeletal plate. Each vacuole opens to the surface by a short, cylindrical excretory canal from 2 to 4μ in length and from 1 to 2μ in diameter. The canal is formed by an inward extension of the pellicle, which is a single homogeneous layer as in *Ostracodinium, Epidinium,* and *Polyplastron.*

Cycle.—In general, the vacuolar cycle of *Ophryoscolex* is similar to that in Epidinium. The resting vacuole (fig. E, *1*) is a large, lentoid vesicle from 20 to 40μ in the antero-posterior diameter and from 15 to 25μ thick. Systole is relatively rapid, lasting from 0.4 to 1.9 seconds. A small vacuole always remains after systole (fig. E, *3*). The fusion of the new accessory vacuoles takes place from 30 to 50 seconds after the end of systole, the rest of diastole lasting from 1 to 5 minutes. The resting period in *Ophryoscolex* is unusually long, normally lasting from 15 to 45 minutes. Occasionally the complete cycle lasts only two minutes.

The osmiophilic granules of *Ophryoscolex,* while common in the general ectoplasm, never show so marked a degree of concentration around the vacuoles as do those of *Epidinium.* This may be correlated with the relative slowness of the vacuolar cycle in *Ophryoscolex* and with the infrequency of concentration of any one vacuole, as well as with the relatively widespread formation of the accessory vacuoles in the ectoplasm. Since the osmiophilic granules are primarily concerned with formation of the accessory vacuoles, the osmiophilic granules also are scattered rather than concentrated near the contractile vacuole.

CONTRACTILE VACUOLES OF OSTRACODINIUM

Ostracodinium monolobum Dogiel 1927 was the species most thoroughly studied in this investigation. *O. gracile* (Dogiel, 1925) and *O mammosum* (Railliet, 1890) were also investigated as a check on the results obtained from *O monolobum*

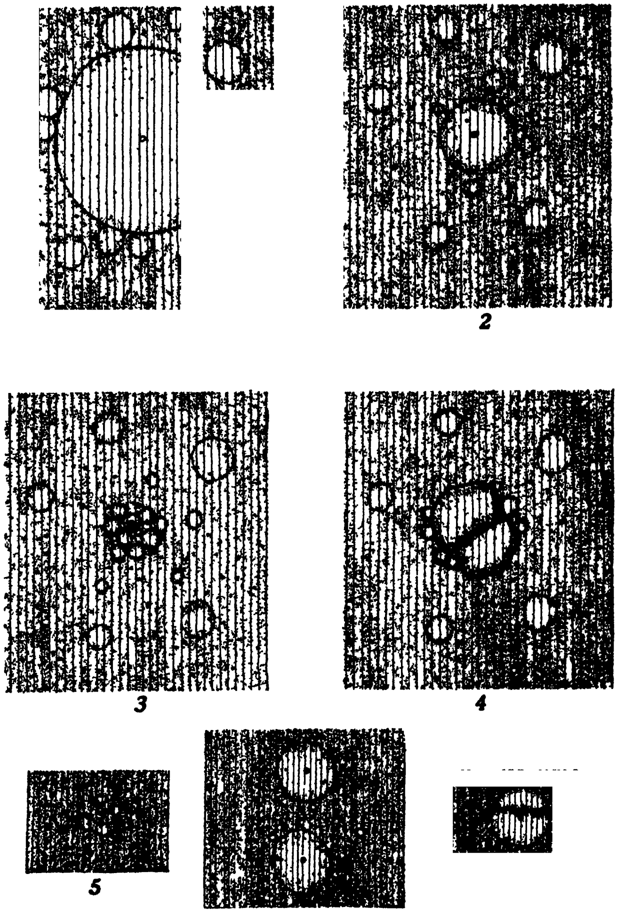

Fig. E Surface view of contractile vacuoles during the pulsatory cycle from individuals stained vitally with neutral red Granules staining with neutral red are arbitrarily drawn larger than the non staining granules Semidiagrammatic, × 1000.

1–4, Ophryoscolex caudatus. 1, resting stage, *2,* systole, *3,* end of systole; *4,* diastole.

5–7, Ostracodinium monolobum. 5, end of systole; *6,* resting stage; *7,* diastole.

There are from two to six contractile vacuoles along the dorsal mid-line of *Ostracodinium.* Two vacuoles are present in *O gracile,* three in *O. mammosum,* and five in *O. monolobum* (pl 15, fig. 5). These vacuoles are adjacent to the left border of the macronucleus, lying in a longitudinal strip of ectoplasm thicker than that of the rest of the body. Fully expanded vacuoles vary in antero-posterior diameter from 4 to 20μ and in dorso-ventral diameter from 3 to 12μ. In species with a relatively large number of vacuoles such as *O. monolobum,* the region of gel around the vacuoles forms a continuous longitudinal strip in the general ectoplasmic sol. This strip is also marked by a concentration of neutral red granules and Golgi granules.

Cycle.—The preliminary rounding of the vacuoles lasts from 8 to 10 seconds, while systole only lasts from 0.8 to 1.9 seconds. The main vacuole contracts until a vesicle about 4μ in diameter remains (fig. E, *5*), and then systole ceases. The neutral red granules retain their position for a short time after systole, so that the former position of the vacuole is marked for a short time by a sphere of colorless, granular ectoplasm surrounded by ectoplasm filled with small accessory vacuoles and with granules stainable with neutral red. The whole vacuolar cycle lasts at least two minutes, the usual period for an actively contracting vacuole being about 2.5 minutes.

The accessory vacuoles in most of the Ophryoscolecidae are formed in a more or less limited region around a particular vacuole and fuse only with it. The vacuoles in *Ostracodinium monolobum,* on the other hand, are so close together that the accessory vacuoles may fuse with either one of two adjacent contractile vacuoles. In some specimens the accessory vacuoles assembled near a canal after systole fuse with the adjacent contractile vacuole instead of forming a new vacuole for the canal. In other specimens two contractile vacuoles may grow so large that they touch and fuse, the double vacuole then becoming detached from one of the canals and emptying through the other. In such an occurrence the only indication of one vacuole is the canal and pore. These variations produced by the extreme crowding of the contractile vacuoles, and also the occasional formation of large accessory vacuoles which may be confused with the true contractile vacuoles, make the correct counting of the contractile vacuoles in fixed specimens of the species difficult and at times impossible.

A layer of osmiophilic granules is found around the vacuole during diastole and shows clearly the formation of accessory vacuoles within it (pl. 17, fig. 35). This vacuole is strikingly similar to the vacuole of *Cycloposthium dentatum* f. *scutigerum* described by Strelkow (1931*a,* pl. 15, figs. 19–20). He interpreted this as a stage in the division of the vacuole. The individual *O. obtosum* from which our figure was made, showed no indication of the multiplication of any onganelles, which marks division; also this stage in fixed material corresponds to the stage of diastole in which growth of the vacuole is accomplished by the formation and fusion of small accessory vacuoles as observed in living material. We conclude that his interpretation is incorrect. The appearance of small vesicles in the osmiophilic region around a contractile vacuole indicates the formation of accessory vacuoles rather than a division of the whole organelle.

CONTRACTILE VACUOLES OF POLYPLASTRON MULTIVESICULATUM

Polyplastron multivesiculatum (Dogiel and Fedorowa, 1925) shows as many as ten vacuoles (fig. B, *1*), a larger number than any of the other Ophryoscolecidae. Four vacuoles are arranged longitudinally along the dorsal mid-line. One vacuole lies just to the left of the anterior vacuole of the dorsal row. The rest of the vacuoles are found under the right side. One is occasionally found between the two main skeletal plates. One pair of vacuoles, arranged longitudinally, lies ventral to the right skeletal plates, and another pair is placed parallel to the first pair and ventral to it. The number of vacuoles present in an individual varies considerably from time to time. Only seven vacuoles were present in one individual with a retracted oral zone. The oral zone opened shortly after the observations were started and the ciliate began to feed. Four more vacuoles appeared in a short time, each of them going through several pulsatory cycles. Pores and excretory canals were found in individuals with less than ten vacuoles in the positions occupied by vacuoles when all were present. These observations were repeated on many other individuals. The variation in the number of vacuoles of *Polyplastron* is dependent, therefore, upon the intake of fluid during feeding. During periods of lessened fluid intake, some of the vacuoles are indicated only by the excretory pores and canals.

The fully expanded vacuole (fig. F, *5*) is relatively small, with an average diameter of about 15μ (ranging from 12 to 30μ), and a dorso-ventral diameter of from 8 to 15μ. These fully formed vacuoles are smaller and more nearly spherical than those of any other genera studied excepting *Ostracodinium.*

Cycle.—The preliminary rounding of the vacuole lasts from 1 to 3 seconds. Systole then starts, lasting from 1.5 to 3.0 seconds, and is usually complete. The accessory vacuoles and the spherules staining with neutral red retain their position during systole (fig. F, *1, 6*), the space formerly occupied by the resting vacuole being filled by the matrix and the non-stainable spherules.

The fusion of accessory vacuoles is complete in from 20 to 60 seconds. The vacuole increases in size for a time without addition of accessory vacuoles, the wall becomes smooth and the vacuole usually becomes elongated in the antero-posterior axis. A concentrated ring of granules assembles at this time around the vacuole (fig. F, *4*) and small accessory vacuoles arise within it and empty into the main vacuole. The spherules forming this ring are not stainable with neutral red. The ring of spherules gradually disappears as the accessory vacuoles form within it. Some of these vacuoles remain intact and later fuse to form the next contractile vacuole. The formation and disappearance of the ring occupies from 30 to 60 seconds.

The total cycle from systole to systole takes from fifty seconds to ten minutes, the average time in the specimens studied being about two minutes.

Most of the accessory vacuoles arise just after systole, replacing the old contractile vacuole. The granules in parts of this region gradually decrease in number and then very suddenly the clear vacuolar fluid is differentiated from the surrounding ectoplasm, which becomes more viscous. Smaller solated regions appear also in the surrounding ectoplasmic gel and small accessory vacuoles are formed in the same manner. All these vacuoles migrate toward the canal and fuse to form the small, irregular vesicle of early diastole. The formation of the small accessory vacuoles in the granular ring in late diastole is not so apparent as that described above as occurring just after systole.

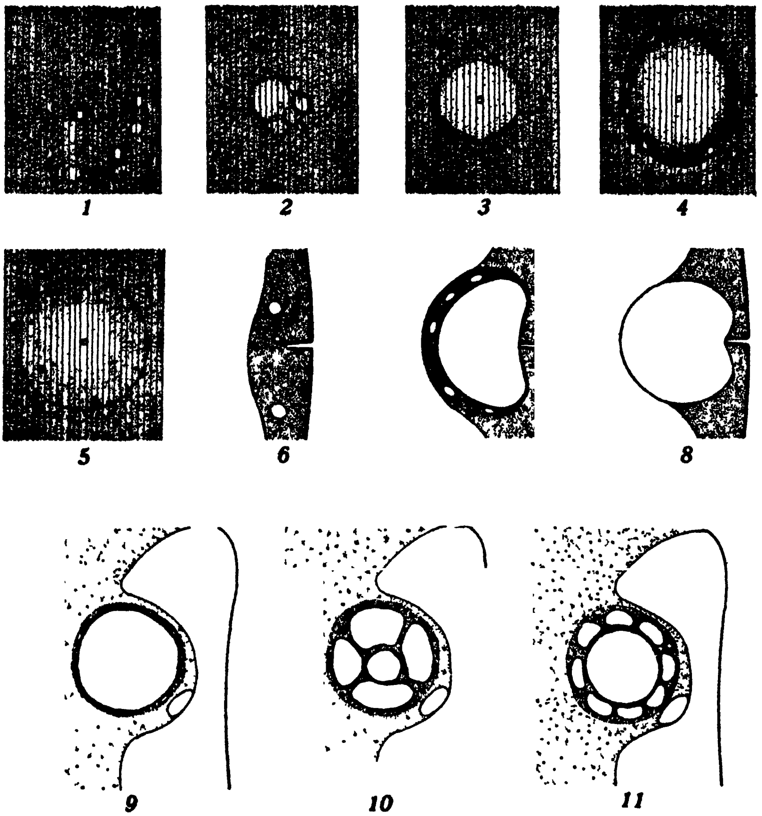

Fig. F. Contractile vacuoles during the pulsatory cycle from live specimens stained vitally with neutral red. Granules staining with neutral red are arbitrarily drawn larger than the non-staining granules. Semi-diagrammatic.

1–8, *Polyplastron multivesiculatum*, × 1000. *1–5*, surface view of anterior dorsal vacuole; *6–8*, side view of the same vacuole in optical section. *1*, end of systole; *2*, beginning of diastole; *3*, end of the first stage of diastole; *4*, second period of diastole; *5*, resting stage; *6*, end of systole; *7*, second stage of diastole; *8*, vacuole rounded just prior to systole.

9–11, *Metadinium medium*, × 500, lateral view of the anterior contractile vacuole in optical section. The anterior end of the macronucleus and the micronucleus are shown in outline. *9*, resting stage; *10*, middle of systole; *11*, late diastole.

At the end of systole, as shown by osmic preparations, the vesicle is replaced by several small vacuoles (pl. 17, fig. 20), around which are clustered a number of relatively large osmiophilic granules. In the next stage, the single small vacuole of early diastole (pl. 17, fig. 21) is surrounded by a layer of very fine osmiophilic granules in which are imbedded a few large granules such as occur in the previous stage. As the accessory vacuoles fuse, these large granules either break up and form this band of smaller granules, or they disappear and are replaced by a new set of small ones. The origin of the smaller granules from the larger ones seems the more reasonable interpretation. This osmiophilic band thickens (pl. 17, figs. 22, 23; pl. 15, fig. 7) and in some specimens reaches a diameter of 5μ. The granular character of this band is apparent only in material impregnated at room temperature (pl. 17, fig. 25). In material impregnated with osmium tetroxide at from 30° to 35° C (pl. 17, fig. 26), the granular zone appears as a homogeneous black band, the only evidence of the original granular structure being the rough outer surface.

In the next stage, small clear vesicles are found in the osmiophilic region (pl. 17, fig. 27). Since this band of osmiophilic granules corresponds exactly to the band of closely packed granules observed during the vacuolar cycle in the live specimens, it is clear that the osmiophilic granules and the granules stainable with neutral red are two entirely different types of cytoplasmic inclusions.

CONTRACTILE VACUOLES OF METADINIUM MEDIUM

Metadinium medium Averinzew and Mutafowa 1914, the only species of the genus found thus far in California cattle, possesses two large, nearly spherical vacuoles beneath the right dorsal surface (fig. A, *2*). These vacuoles are half imbedded in two large dorsal hollows of the macronucleus, one near the anterior end and one near the posterior end.

The excretory canal is from 8 to 10μ long (pl. 17, fig. 32) and opens by means of a small, circular, outer excretory pore, from 3 to 5μ in diameter. The pellicle of *Metadinium,* as well as that of *Eudiplodinium,* is in two layers, a thick inner striated layer, and a thin, outer clear layer. An inward extension of the clear layer alone forms the wall of the excretory canal.

The resting vacuoles (fig. F, *9*) of *Metadinium medium* are large and nearly perfect spheres, from 25 to 35μ in diameter. A narrow, densely granular rim from 1 to 3μ in thickness surrounds each vacuole. Small flattened accessory vacuoles are imbedded in this region. A few granules stainable with neutral red are found in the rim, but the majority of the granules could not be stained with any of the vital dyes used in this investigation. None of the granules in the vacuolar region exhibit Brownian movement at this time, demonstrating that it is a relatively stiff gel. The general ectoplasm has fewer granules than the vacuolar region, but a large proportion of these granules are stainable with neutral red, so that in an individual stained with this dye the pale greenish gray vacuolar region is bounded by myriads of brilliant red granules. The granules in the general ectoplasm show marked Brownian movement, the sol state thus indicated differing markedly from the gel state of the granular band around the vacuoles.

Cycle.—The main vacuole contracts in a leisurely fashion (fig. F, *10*), taking from 21.2 to 26.0 seconds for the completion of systole. The small accessory vacuoles in the granular rim expand rapidly and fuse together as the main vacuole contracts. There is at the same time a definite flow of granules from the general cytoplasm into the vacuolar region, so that the total diameter of the vacuolar region (vacuole plus granular rim) remains unchanged. Systole is usually complete and the remainder of the wall of the old vacuole forms the closure membrane over the inner excretory pore.

The osmiophilic granules in *Metadinium* form a heavy band around the contractile vacuole and are also scattered evenly throughout the whole ectoplasm. Only the granules in the band around the vacuole appear to be concerned directly with the vacuolar cycle. The osmiophilic rim can be demonstrated in osmic preparations in all stages of the cycle. This confirms the permanency of the granular rim as observed in living specimens and emphasizes the contrast to the granular region, which is present in *Eudiplodinium* only in diastole.

STRUCTURE OF THE CONTRACTILE VACUOLE IN THE OPHRYOSCOLECIDAE

Pore.—The presence of a permanent pellicular pore has been denied for many ciliates. Khainsky (1910) described instead a rhythmic rise and fall of the pellicle over the vacuole of *Paramecium* during the pulsatory cycle and gave the name "papilla pulsatoria" to the portion of the pellicle involved. This activity was described entirely from fixed material. Haye (1930) described a similar appearance in *Polyplastron multivesiculatum* and *Ostracodinium gracile* and like Khainsky found no excretory pores or canals. The observations reported in this paper, however, show that the papilla pulsatoria is never present in living material and that the rise and fall of the pellicle over the vacuole as figured by Haye is caused by the processes of fixation, imbedding, and sectioning.

The papilla pulsatoria described by Taylor (1923) in *Euplotes* is a thin protoplasmic membrane sealing a permanent pore in the hard, rigid pellicle. The drawings published by Bretschneider and Hirsch (1927) of the papilla pulsatoria in *Balantidium giganteum* indicate a similar type of structure. These structures are entirely different from the large invaginations and evaginations of the pellicle of *Paramecium* described by Khainsky and should not be included under his term.

The excretory canal and the inner and outer excretory pores are permanent pellicular structures in the Ophryoscolecidae. The canal is rigid at all times and shows no traces of sphincters like those described by Strelkow (1931*a*) in the Cycloposthiidae. The inner pore is closed at all times, except during systole, by a thin protoplasmic membrane, formed from the remnants of the wall of the old vacuole. The inner excretory pore of the contractile vacuoles in the Ophryoscolecidae is similar to the permanent opening in the pellicle of *Euplotes,* sealed except during systole by a delicate protoplasmic membrane. Zenker (1866) gave a similar interpretation for the vacuole of several ciliates.

Vacuolar wall.—The presence of a permanent membrane bounding the contractile vacuole has been the subject of much dispute, but since the observations of Wrzesniowski (1869) it has been generally admitted that in those vacuoles which are formed by the fusion of newly arising accessory vacuoles, the boundary membrane is only a temporary differentiation of the cytoplasm. Taylor (1923), on the basis of his observations on *Euplotes,* corroborates this view.

The wall surrounding the contractile vacuole and the accessory vacuoles of the Ophryoscolecidae is temporary, disappearing during systole and re-forming anew as each new accessory vacuole is formed. The wall, like that in *Euplotes,* is not a membrane in the structural sense, since only the internal surface of the wall is sharply differentiated and the outer surface grades indistinctly into the surrounding ectoplasm.

Kraschenninikow (1929) and Haye (1930) described a definite membrane around the contractile vacuoles in several genera of the Ophryoscolecidae. Kraschenninikow claims that the membrane is osmiophilic. Haye described small granules imbedded in the membrane and stated that these are lipoidal in nature. He also described the accessory vacuoles as arising in this membrane, appearing in his drawings as little blisters. The presence of a definite membrane in the vacuoles of the Ophryoscolecidae is due entirely to fixation and is the result of partial fusion of the granules immediately surrounding the vacuole, giving the appearance of a heavy, vacuolated membrane. In *Eudiplodinium,* the form on which Kraschenninikow based his conclusions, the boundaries of the vacuoles are relatively inconspicuous in material impregnated with osmium tetroxide, while the outer boundary of the vacuolar region is very sharp. Thus Kraschenninikow mistook the outer limit of the heavy granular vacuolar region surrounding the vacuole proper for the vacuolar membrane.

The consistency of the cytoplasm forming the vacuolar wall is usually described as being slightly more viscous than the adjacent cytoplasm (Taylor, 1923; Howland, 1924). This conclusion is based both on micro-dissection experiments and on the fact that the granules next to the vacuole do not show Brownian movement. A similar narrow rim of viscous material adhering to the vacuole is present in the Ophryoscolecidae.

The viscous layer apparently solates at times and allows the accessory vacuoles to fuse. The ability of accessory vacuoles to fuse is dependent on the phase of the vacuolar cycle. Some of the accessory vacuoles formed in the extremely fluid vacuolar region of *Eudiplodinium* during the end of systole bump together quite forcibly under the influence of the localized cytoplasmic currents in this region but show absolutely no tendency to fuse at this time. After systole is entirely completed, however, these accessory vacuoles fuse with one another at the slightest touch. Howland (1924) notes a similar phenomenon in *Amoeba.* Therefore, although the immediate cause of the fusion of two contiguous vacuoles is their contact, contact alone is insufficient to cause fusion unless another factor is operative. This necessary factor is probably the solation of the delicate, indeterminate gel envelope forming the vacuolar wall.

Vacuolar region.—Differentiations of cytoplasm around the contractile vacuoles have been noted in many ciliates. Fauré-Fremiet (1925), Fortner (1926), and others have considered this region to be intimately concerned with the phenomena of vacuolar cycles and have called it the "Nephridialplasma." *Spirostomum* (Day, 1930; Haye, 1930), and *Blepharisma* (Haye, 1930), etc., show no specialized region around the contractile vacuoles.

The position of the main vacuoles is permanent in all the Ophryoscolecidae and the formation of accessory vacuoles takes place in a more or less limited area, the degree of localization varying in the different genera. No region of cytoplasm around the vacuole is visibly differentiated from the rest of the ectoplasm in either *Ophryoscolex* or *Epidinium.* The vacuoles of *Ostracodinium* lie in a longitudinal band of gelated ectoplasm, solation occurring only in localized regions around an active vacuole. There is a temporary differentiation of ectoplasm around the vacuoles of *Polyplastron* during diastole. A very definite band of ectoplasm forms around the vacuoles of *Eudiplodinium* during diastole. In *Metadinium* a similar band is present during the whole cycle. The formation of the new vacuoles in both of these genera is entirely restricted to this vacuolar region. The material of these specialized regions is clearly derived from the general ectoplasmic material and in particular represents a concentration of osmiophilic granules. Thus within the Ophryoscolecidae all degrees of concentration of vacuolar materials are represented and the genera may be arranged in an intergrading series extending from *Ophryoscolex* with a very slight concentration of osmiophilic granules around its vacuoles during diastole, up to *Metadinium* with a permanent band of osmiophilic granules around its vacuoles. This series also represents a gradation in the formation of accessory vacuoles. These vesicles in *Ophryo-*

scolex form in most of the ectoplasm and arise as very minute vesicles below the range of visibility and can be seen only when fully formed. In *Epidinium* the formation of accessory vacuoles is restricted to the dorsal side; in *Ostracodinium* the formation is restricted to a narrow dorsal strip and the vesicles are visible from their earliest stages; in *Polyplastron* they form in a similar restricted region, and in later diastole the last of the accessory vacuoles form in a granular band around the vacuole; finally in *Eudiplodinium* and *Metadinium* they form only in the restricted granular region and are relatively large even in their earliest stages. Thus where there is no localization of formation of accessory vacuoles, there is no concentration of granules around the contractile vacuoles; where there is a localization of vacuolar formation there is a great concentration of granules in the vacuolar region. This correlation of osmiophilic granules and accessory vacuole formation definitely indicates that these granules play an important rôle in the vacuolar cycle.

ORIGIN OF THE ACCESSORY VACUOLES

The differentiation of small accessory vacuoles from the cytoplasm is one of the fundamental problems in a study of the contractile vacuoles. The origin of the accessory vacuoles has been described in other ciliates either as a fusion of pre-existing, but invisible, vacuoles of the ectoplasm, or as an entirely new concentration of fluid with a subsequent differentiation of the vesicle from the cytoplasm. Taylor (1923) showed that the granules of *Euplotes* oscillate in small, fluid-filled vacuoles. He states that in some the granules may dissolve and thus give rise to the small, clear accessory vacuoles (his group V_3). He also shows the possibility of a *de novo* origin.

The accessory vacuoles in *Metadinium, Polyplastron, Eudiplodinium,* and *Ostracodinium* arise as relatively large vesicles and can be made out clearly in all stages of formation. There is first a localized solation of the ectoplasm, then a gradual disappearance of granules in the solated portion, and finally a very sudden differentiation between the clear vacuolar fluid and the surrounding ectoplasm, producing the appearance of a miniature explosion as referred to by Day (1927). There is strong evidence for the solution of the osmiophilic granules in the vacuolar fluid at this time, particularly in *Eudiplodinium* and *Metadinium.* The evidence has been summarized in connection with the description of the osmiophilic granules of *Eudiplodinium.* The accessory vacuoles in these forms do not arise by fusion of ultramicroscopic alveoli but arise *de novo* as relatively large vesicles which are first visible as solated portions of the ectoplasmic gel, the differentiation of the vacuolar contents from the protoplasm being only the end of the whole process of formation. The accessory vacuoles of *Epidinium* and *Ophryoscolex* form as minute submicroscopic vesicles and therefore their method of origin cannot be directly demonstrated. There is, however, no evidence of an alveolar structure of the ectoplasm in these two genera, and since the *de novo* origin is certain in the other four genera investigated, a like origin of the accessory vacuoles in *Epidinium* and *Ophryoscolex* is probable.

The accessory vacuoles normally form in a regular manner, fuse to form one of the contractile vacuoles, and their fluid is thus forced outside of the ciliate. Abnormal specimens, either kept on a slide too long or injured in other ways, show the formation of many irregular vacuoles in all regions of the ectoplasm and in such numbers that only a few of them are eliminated by fusion with the contractile vacuole. Vacuolation is a fundamental property common to all the ectoplasm, but its action is restricted in normal individuals to a relatively narrow region. Thus the formation of a fluid center which results in the localized accumulation of fluid in a vacuole (Dixon, 1920; Taylor, 1923) is accomplished through the action of a specialized region of the ectoplasm and in that sense the contractile vacuolar system is a permanent organelle.

SOURCE OF THE VACUOLAR FLUID

The path of the water through the protozoan cell has excited little interest compared to the attention paid the contractile vacuole itself. Hartog's theory (1888) involves the assumption that the whole surface membrane is permeable to the water of the surrounding medium; also that the intake of water of any given specimen is solely dependent on the concentration of this medium. Degen (1905) and others have accepted his view. Recently the permeability of the whole outer membrane has been questioned to some extent. Taylor (1923) showed that the accessory vacuoles most commonly arose near food vacuoles and on this basis considered the main point of entry to be through the oral region. Eisenberg (1925) considered that in *Paramecium caudatum* most of the water entered by way of the protoplasm bordering the peristome.

There is definite evidence that the pellicle of the Ophryoscolecidae is relatively impermeable. The penetration of vital dyes is very rapid during active feeding, the maximum depth of staining being reached in less than half a minute. When the oral region of one of the Ophryoscolecidae is closed, more than half an hour elapses before its cytoplasmic granules are distinctly colored. Pulsation of any of the vacuoles in a specimen with a closed oral region is rare, but is common in actively feeding specimens. In *Polyplastron* several of the vacuoles are present only when the individual is actively feeding. It is clear that very little of the excess water and other materials can be eliminated through the general surface of the body. It is easily shown by direct observation that very little, if any, of the extra fluid taken in during feeding leaves through the anal opening.

All this evidence indicates that the pellicle covering the general surface of the body is relatively impermeable. The contractile vacuoles alone eliminate most of the fluids. Most of the fluid excreted by the contractile vacuoles enters through the cytostome. One function of the contractile vacuole, therefore, is to eliminate the excess water taken in with the food materials. At the same time, this water, as it is eliminated, serves as the vehicle for dissolved materials such as katabolic wastes.

VACUOLAR CYCLE

Resting stage.—Many contractile vacuoles after attaining full size show a more or less marked period in which no appreciable change either in size or form occurs. This stage is particularly prominent in the Ophryoscolecidae, in which the contractile vacuoles are only occasionally active. Any assemblage of osmiophilic granules is completely absent or at a minimum at this time and the surrounding ectoplasm is firmly gelated. This period of the pulsatory cycle thus presents a marked contrast to diastole and systole which are both accompanied by so many striking cytoplasmic changes. The lack of concentration of osmiophilic granules near the contractile vacuoles during the inactive part of the pulsatory cycle is the condition to be expected if these granules are intimately concerned with the formation of contractile vacuoles.

Systole.—The stimulus for systole is unknown. Taylor (1923) showed that the vacuole of *Euplotes* must touch the excretory pore before systole can occur and concluded that this is the stimulus for systole. The vacuoles in the Ophryoscolecidae, however, are in close contact with the pore during the latter half of diastole and during the resting period. Contact of the vacuole and the pore is therefore not a stimulus for systole in the Ophryoscolecidae. It is possible that in *Euplotes* the correlation between contact and systole is only a coincidence. The size of the vacuoles has nothing to do with commencement of systole. Systole may take place at any time after the first fusion of the accessory vacuoles and a resting vacuole, which thereupon suddenly contracts without any increase in size. Botsford (1926) and others have made similar observations in other Protozoa. Therefore while it has been shown definitely in several forms that systole is immediately preceded by a solation of the membrane closing the excretory pore and of the cytoplasm immediately around the vacuole, the stimulus behind that phenomenon is still unknown.

Many theoretical explanations of systole have been advanced, but some of them are contrary to observed facts and none accounts completely for all the observable phenomena. It is also probable, if not certain, that no single explanation can be applied to the vacuoles of all Protozoa. For example, Dixon's theory (1920) of the contractile vacuole assumes that there is a gel structure surrounding the contractile vacuole throughout the whole cycle and that the energy necessary for the expulsion of the vacuolar fluid comes from the elasticity of this postulated gel. Actually, systole of the contractile vacuoles of many of the Protozoa is accompanied by signs of marked solation of the surrounding cytoplasm. Taylor (1923) showed that the membrane closing the excretory pore in *Euplotes* (his papilla pulsatoria) reverts to a sol condition and ruptures at the beginning of systole, and he implies a solation of the whole region around the vacuole.

Systole of the contractile vacuoles in the Ophryoscolecidae occurs only when the surrounding region is solated. During this solation, the membrane closing the inner excretory pore is dissolved or so weakened that it ruptures. This is exactly opposite to the conditions postulated by Dixon (1920) in his theory of the mechanism of vacuolar action, and his theory cannot be applied either to the Ophryoscolecidae or to *Euplotes.*

The problem of systole is further complicated by the occasional occurrence of partial systole. This is a widespread phenomenon and has been reported in *Euplotes* by Taylor (1923), in various species of *Amoeba* by Botsford (1926), Howland (1924), Howland and Pollack (1927), and in *Actinosphaerium* by Howland and Pollack (1930). Howland and Pollack present evidence to show that partial systole in *Amoeba dubia* is caused by strong cytoplasmic currents which pull the vacuole away from the temporary pore. This was observed in the present investigation in *Ophryoscolex, Ostracodinium, Polyplastron,* and *Eudiplodinium.* Many incomplete systoles were observed, however, in which the remnant of the contractile vacuole remained attached to the excretory pore. In the latter situation, the most we can say is that the forces which produce systole cease to be effective.

The force utilized in expulsion of liquid from the vacuoles is usually ascribed to surface tension, or intracytoplasmic pressure, or both. The theory of systole by intracytoplasmic pressure has been attacked at various times. The chief objection has been that in forms with more than one vacuole, systoles of the vacuoles are not synchronous, although the intracytoplasmic pressure is presumably the same around all the vacuoles. This objection is not valid in the Ophryoscolecidae and probably not in other forms, since the pore of one vacuole may be open while the pores of the other vacuoles are securely plugged by the gelated protoplasmic membrane. The forces of surface tension and of intracytoplasmic pressure are held in check except during systole by the closing membrane.

Diastole.—The diastole of the contractile vacuole is usually considered to involve first an accumulation of osmotically active materials, soluble crystalloid materials, according to Dixon (1920), or katabolic products, according to Taylor (1923), followed by a migration of water to this region. Nassonow (1924) put forth the hypothesis that the osmiophilic region around the vacuoles of certain ciliates is responsible for the accumulation of this osmotically active material. He homologizes the action of this osmiophilic material with that of the Golgi material of secretory cells. Bojewa-Petruschewskaja (1928) showed that the osmiophilic layer around the vacuole of *Balantidium* is demonstrable only during diastole.

Diastole in all genera of the Ophryoscolecidae is in two parts—first the formation and fusion of the accessory vacuoles, next the slow growth of the vacuole to full size in which no more accessory vacuoles fuse with the main vesicle. The cytoplasm immediately around the accessory vacuoles becomes more and more viscous during the fusion process and by the time the second stage of diastole appears it is completely gelated. The osmiophilic granules appear in greatest number in this first period of diastole and then rapidly diminish in number in the second period.

The two parts of diastole demonstrated morphologically in the contractile vacuoles of the Ophryoscolecidae correspond to the two parts of diastole as postulated by present theories of vacuolar action. The early formation of accessory vacuoles and the accumulation of osmiophilic granules represent the localization of osmotically active materials, the passive growth of the vacuole, and the loss of osmiophilic granules in late diastole corresponding to the accumulation of water brought about by the osmotically active materials.

STRUCTURE OF THE ECTOPLASM

The ectoplasm is composed of a clear, optically homogeneous matrix in which many small granules are imbedded. Most of the ectoplasm in the genera observed is normally in a sol state at all times, but the ectoplasm which lies near the contractile vacuoles undergoes periodic sol-gel changes. From direct observation the gel appears to be of the same consistency throughout, but the observed movement of granules during systole cannot be explained on this basis. There is a distinct flow of granules into the vacuolar region of *Eudiplodinium* as the vacuole contracts, and these granules are of one type only, the osmiophilic granules which cannot be stained vitally with neutral red. The granules come from the surrounding gelated ectoplasm in which they are intermingled with the granules staining with neutral red. Thus the non-stainable granules migrate into the vacuolar region while neutral red granules lying next to them remain fixed. A similar differentiation of movements between the two types of granules is found in *Ostracodinium* and *Polyplastron*. The movements of small accessory vacuoles in these latter genera are similar to the movements of the granules. Some of the accessory vacuoles migrate toward the accessory canal shortly after systole while adjacent vacuoles show no movement at all.

These observed differences in the movements of adjacent granules or of adjacent accessory vacuoles may be explained as resulting from the presence of a coarse, viscous reticulum in which are imbedded the granules of the vacuome and some of the accessory vacuoles, the interstices between the meshwork being filled with fluid of relatively low viscosity which contains part or all of the granules that do not react to neutral red and also some of the accessory vacuoles. Thus the non-stainable granules are free to move along with the fluid matrix while the neutral red granules are held by the viscous reticulum. This meshwork is relatively coarse and the pores must be at least 0.2μ in diameter in order to allow the granules to migrate freely through them. The reticulum is much larger than the postulated brush-heap structure of a gel and is a structure formed by the gel rather than being the structure of the gel itself.

A viscous reticulum as described above fits in with the observed vacuolar cycles. The ectoplasm around the resting vacuoles of *Ostracodinium* is apparently a solid gel showing no movements of the granules. Just before systole the granules of this region begin to show Brownian movement which is particularly marked in the osmiophilic granules. This marks the first appearance of the coarse viscous mesh and the less viscous phase containing the osmiophilic granules. The homogeneous gel around the resting vacuole thus changes to a viscous reticulum containing a solated phase in its meshes sufficiently pliable to allow the vacuole to become spherical. As the vacuole contracts, the fluid phase fills the space left by the retreating walls of the contractile vacuole. The granules stainable with neutral red thus remain outside the completely solated vacuolar region for a time while the other granules migrate in. Shortly after systole is complete, the neutral red granules also move into the completely solated vacuolar region, indicating that the gelated mesh has also become solated. This interpretation applies also to *Polyplastron*. The gelated reticulum around the vacuoles of *Eudiplodinium* is apparently more permanent, since neutral red granules never get into the vacuolar region. The movements of the granules indicate its presence throughout systole and during the first part of diastole. During late diastole and the resting stage, lack of Brownian movement of all the granules around the vacuolar region indicates that the gel is again homogeneous.

The presence of a viscous mesh, interstices of which are filled with the fluid phase, is thus in accord with the observed vacuolar cycle and is an hypothesis which adequately accounts for differences between the observed movements of the vacuome granules and the osmiophilic granules. Mast (1926) describes small fluid vacuoles in the gelated ectoplasm of *Amoeba*. The reticulum described above is similar to Mast's concept with the exception that the fluid phase is continuous rather than in discrete vacuoles as in *Amoeba*.

IDENTIFICATION OF GOLGI MATERIAL IN CILIATES

Various criteria have been suggested by different authors to facilitate the identification of Golgi material. Hirschler (1927) stressed the importance of positive identification of mitochondria in order to eliminate confusion of Golgi material with the chondriome. Bowen (1928*b*) suggested in addition that consistent impregnation by both the silver and osmic methods would be good proof. He also stressed resistance to bleaching in osmic impregnated material as a character of true Golgi material. Hall (1931) used as criteria the above staining reactions and in addition stressed the idea that the Golgi material is probably quite similar in morphology and distribution in all Protozoa. He therefore restricts the term Golgi material to those inclusions which can be demonstrated consistently by the standard methods and which are similar in form and intracytoplasmic distribution in all Protozoa.

The morphology of the osmiophilic materials in ciliates is still a matter of dispute. The great majority of workers in this field describe small granules as Golgi material, but a few, led by Nassonow (1924), homologize either the wall of the contractile vacuole or a shell around the wall of the contractile vacuole (Strelkow, 1931*b*) with Golgi material.

The workers demonstrating a solid membrane have used the warm impregnation method advocated by Nassonow (1924). Hirschler (1924) showed that this warm method tends to produce over-impregnation, and in closely packed spherules the osmic precipitate spreads and obscures the true granular nature of the region. He advocates a cold method of impregnation to eliminate this source of error. Impregnation of the Ophryoscolecidae strikingly bears out this view. The heavy black membrane around the vacuole visible in material treated by the warm impregnation method (pl. 17, fig. 26) is shown by cold impregnation to be granular (pl. 17, fig. 25) and *this granular composition of the vacuolar region is seen in living material.* The figures showing solid impregnation of the vacuolar walls of *Chilodon* and *Dogielella,* published by Nassonow (1925), of *Balantidium* by Bojewa-Petruschewskaja (1928), and of the Cycloposthiidae by Strelkow (1931*b*) indicate a marked granular roughening on the outer margin of the osmiophilic layer, giving evidence that these solid black membranes are the result of over-impregnation of an originally granular zone. The blackened membranes or walls of vacuoles are therefore interpreted as being heavy aggregations of osmiophilic granules rather than solid structures. The variations in the impregnation of regions around the contractile vacuoles in different ciliates may then be explained as representing a difference in the localization of the formation of the contractile vacuole. A series extending from practically no aggregation of osmiophilic granules to a very heavy aggregation around the vacuoles has been demonstrated in the Ophryoscolecidae and has been shown to be correlated with the degree of localization of the formation of the contractile vacuoles. The main difference between such forms as *Spirostomum* (Hirschler, 1924*a*) and *Metadinium* is that in the former genus the osmiophilic granules are scattered more or less uniformly throughout the cytoplasm, while in the latter many are concentrated around each vacuole.

Certain granules have been described, however, which are obviously not to be considered as homologous with the granules discussed above. Richardson and Horning (1931) showed that the pyriform granules described by King and Gatenby (1926) in *Opalina* are not Golgi granules. The macronuclear knobs of *Stentor* described by Park (1929) also must be excluded.

The criterion of constancy of morphology and distribution in all Protozoa is a logical one and appears to be fairly valuable unless it is construed so strictly as to rule out any possibility of a cyclical change in morphology or in distribution of the granules. Hall applies this criterion very strictly, sometimes selecting the true Golgi material from other blackened material merely because of greater frequency of occurrence. Such a demand that Golgi material be absolutely constant in distribution and morphology would rule out the Golgi material of gland cells which show marked cyclic changes (Bowen, 1928*b*). Even allowing for cyclic changes, the criterion of constancy of form is valuable only in a negative way. The Golgi material usually accepted as such in the Protozoa is granular in form. This helps to rule out membranes, macronuclear knobs, or parabasal bodies of flagellates, but does not help to distinguish true Golgi granules from other cytoplasmic granules.

Granules which satisfy the criteria of staining reactions but which differ in other properties have been demonstrated in various ciliates. Hall (1931) showed that the granules vitally stained with neutral red may be subsequently impregnated by exposure to osmium tetroxide solution for from 3 to 4 days. Peschkowskaja (1928) showed that the osmiophilic granules of *Climacostomum* could be demonstrated vitally with neutral red, but also showed that neutral red reveals at the same time many granules which are not osmiophilic. In the Ophryoscolecidae, the osmiophilic granules and the granules demonstrable with neutral red are two entirely separate sets of cytoplasmic inclusions. A puzzling phenomenon found in the Ophryoscolecidae may be included here. The only granules in *Eudiplodinium* which can be impregnated with osmic acid are those found in the vacuolar region. It was shown from a study of living material that this region is composed of granules from the surrounding ectoplasm. Therefore these granules, as they assemble in the vacuolar region, undergo some chemical change or, more likely, some physical change which permits of their impregnation by the osmic technique. It is evident that, in ciliates, Golgi material identified by means of staining reactions includes many granules similar in a general way but differing in various details of their staining reactions or in solubility.

It is somewhat questionable then to homologize granules on the basis of their reaction to osmic or silver techniques and it is difficult to homologize them with the Golgi material in the Metazoa. *On the basis of the criteria now available,* however, the osmiophilic granules described in the Ophryoscolecidae are considered to be homologous with the osmiophilic granules described as Golgi material in various other ciliates. They are small granules which can be selectively impregnated by means of the silver or osmic techniques and can be separated from the other cytoplasmic granules, such as mitochondria and glycogen, by appropriate staining methods. The granules are usually found throughout the ectoplasm. They also show a cyclic aggregation around the contractile vacuoles, which is comparable to the changes occurring in the Golgi material in gland cells. Since they do not stain with neutral red, they are not strictly comparable to the vacuome granules described by Hall (1931) in *Stylonychia* and by Dunihue (1931) in *Paramecium.*

The vacuolar region described in the Ophryoscolecidae is strikingly similar to the Golgi region of secretory cells. Both regions are specialized parts which show marked cyclical changes in connection with the expulsion of material from the cell. The cytoplasmic components found in the Ophryoscolecidae and their observed activity correspond more nearly to the views on the activity of Golgi material expressed by Nassonow (1926) and by Bowen (1928*a*) than to the views of Parat (1928). There are three definite types of cytoplasmic components in these ciliates, the Golgi granules, the vacuome, and the chondriome. The Golgi granules can be identified in several of these ciliates when alive, because of the concentration of these granules in the vacuolar region. The findings agree thus far with those of Nassonow and Bowen. The Golgi granules contribute directly to the formation of the accessory vacuoles while neither the vacuome nor the chondriome in these ciliates shows any relation to the vacuolar cycle. The vacuolar region in these ciliates therefore does not conform in this respect with either Bowen's or Parat's interpretation of the function of the Golgi material.

The vacuolar region found in these ciliates shows definite evidence of the elimination of materials by means of the vacuolar fluid and corresponds to the secretory region or "region of Golgi" in gland cells. The nature of the materials eliminated by the vacuolar region was not determined in this investigation. Since, however, the pellicle in the Ophryoscolecidae has been shown to be relatively impermeable and since the vacuolar system is the only demonstrable path by which materials are constantly being passed to the exterior, it is likely that the katabolic wastes of these ciliates are eliminated by this organelle rather than by direct diffusion through the pellicle.

SUMMARY

1. The cycle of the contractile vacuoles was studied in both living and fixed material in the following genera of the Ophryoscolecidae: *Ophryoscolex, Epidinium, Ostracodinium, Polyplastron, Eudiplodinium,* and *Metadinium.*

2. The ectoplasm of these forms is very granular with no alveoli or vacuoles except those found in connection with the contractile vacuoles.

3. There is a permanent excretory pore for each vacuole in this group of ciliates. It opens into a canal formed by the pellicle. The inner end of the canal is closed by a thin membrane except during systole. The wall of the vacuole is a temporary structure. It is never impregnated by either the osmic or the silver techniques.

4. The contractile vacuole in all genera is formed by the coalescence of small accessory vacuoles. The accessory vacuoles arise *de novo* in small solated portions of the ectoplasm. The contractile vacuoles in *Ophryoscolex* and *Epidinium* arise in the whole ectoplasm while those of *Ostracodinium* are restricted in origin to a narrow dorsal strip of the ectoplasm. The accessory vacuoles of *Eudiplodinium* and *Metadinium* are formed only in a sharply defined granular region around the contractile vacuole.

5. The vacuolar fluid is mainly derived from excess fluid taken in by the cystostome during feeding. The general pellicular covering of the body is relatively impermeable and admits little if any fluid. Excretion by diffusion through the pellicle is unlikely.

6. The cycle of the contractile vacuole in all these genera is in three parts: the resting period, systole, and diastole. The resting period is marked by gelation of the surrounding ectoplasm and is a completely static stage. Systole is initiated by a solation of the surrounding ectoplasm and rupture of the membrane over the pore. Systole may be complete or only partly complete. Contact of the contractile vacuole with the excretory pore does not initiate systole. The closure membrane is re-formed from a remnant of the wall of the old contractile vacuole. The early part of diastole is marked by the formation and fusion of accessory vacuoles to form the new contractile vacuole which then becomes attached to the excretory pore. The surrounding cytoplasm then reverts to the gel state and a period of growth takes place. When this ceases, the resting stage commences.

7. Mitochondria, glycogen granules, granules stainable with neutral red, and osmiophilic granules were identified. The osmiophilic granules and the granules stainable with neutral red are interpreted as being the Golgi material and the vacuome, respectively.

8. Osmiophilic granules accumulate around the contractile vacuole during the early part of diastole and then are gradually reduced in number. This

granular vacuolar region can be noted in living material. The formation of accessory vacuoles in these granular regions involves a solution of these granules in the vacuolar fluid. This represents a possible method for the elimination of katabolic wastes.

9. Neither the vacuome nor the chondriome show any relation to the pulsatory cycle.

10. The ectoplasm around the vacuolar region during systole shows evidence of the presence of a coarse, viscous reticulum in which are embedded the granules of the vacuome and with the interstices filled with fluid ectoplasm in which the osmiophilic granules lie. The osmiophilic granules are thus free to move into the vacuolar region, while the viscous reticulum retains the vacuome granules.

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EXPLANATION OF PLATES

All whole mounts and sections, unless otherwise noted, were fixed in Champy's fluid and then impregnated with osmium tetroxide at from 20° to 24° C for a period of from 16 to 21 days, then bleached 48 hours in turpentine. The sections were cut 5μ thick. All drawings were made with the aid of a camera lucida.

PLATE 15

Photomicrographs by Mr. J. E. Gullberg.

Fig. 1. *Epidinium caudatum,* × 550. Dorsal view of the posterior vacuole in diastole.

Fig. 2. *Ophryoscolex caudatus,* × 900. Cross-section at the level of the posterior circlet of vacuoles. Da Fano silver impregnation.

Fig. 3. *Metadinium medium,* × 650. Parasagittal section of the anterior vacuole. This vacuole is near the end of diastole. Compare with pl. 16, fig. 31.

Fig. 4. *Epidinium caudatum,* × 550. Right lateral view of whole mount. An over-impregnated specimen showing complete blackening of the vacuoles.

Fig. 5. *Ostracodinium monolobum,* × 550. Left lateral view of whole mount. All five vacuoles are in the resting stage and are surrounded by minute accessory vacuoles. Compare with pl. 17, fig. 42.

Fig. 6. *Ophryoscolex caudatus,* × 550. Frontal section showing a pair of vacuoles in diastole.

Fig. 7. *Polyplastron multivesiculatum,* × 550. Cross-section at the level of the anterior ventral vacuole.

Fig. 8. *Eudiplodinium maggii,* × 650. Right lateral view of whole mount. Both vacuoles are in the early stage of diastole.

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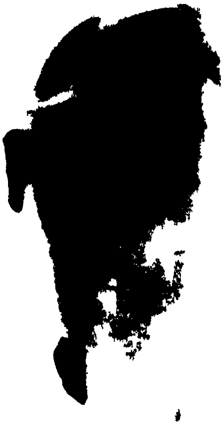

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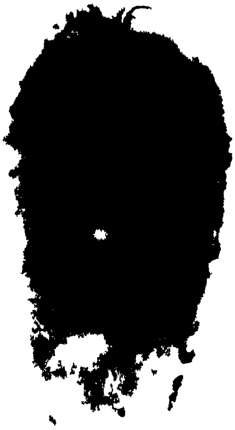

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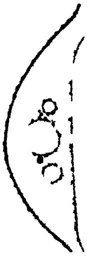
PLATE 16

Figs. 9–15. *Ophryoscolex caudatus*, × 1500. Fig. 9, beginning of systole, cross-section; fig. 10, diastole, longitudinal section; fig. 11, diastole, longitudinal section; fig. 12, diastole, cross-section. Da Fano silver impregnation; fig. 13, diastole, cross section; fig. 14, diastole, cross-section. The large glycogen granules with dark centers are shown in addition to the osmiophilic granules. Osmic impregnation, stained with Sudan III; fig. 15, resting stage, longitudinal section.

Figs. 16–19 *Epidinium caudatum*, × 1500, whole mounts. The posterior vacuole in dorsal view showing in addition to the vacuole the permanent excretory pore and pellicular striations. Fig. 16, diastole; fig. 17, late diastole; figs. 18–19, resting stages.



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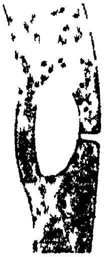

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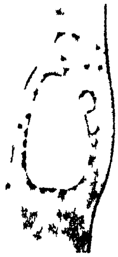

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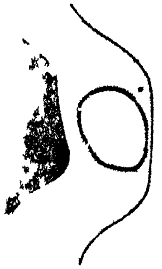

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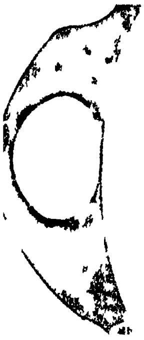

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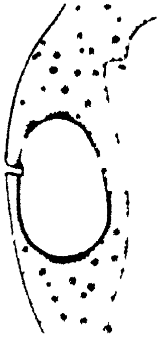

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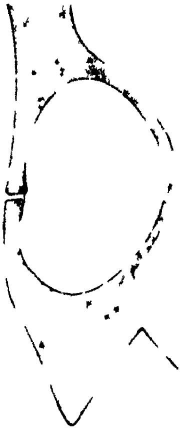

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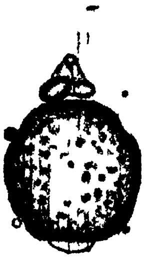

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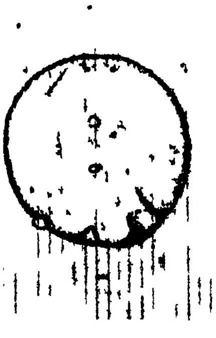

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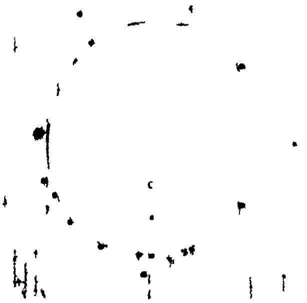

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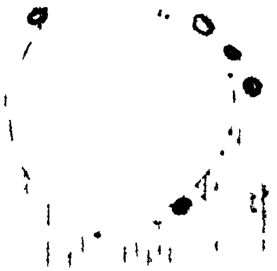

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PLATE 17

Figs. 20–28, 33. *Polyplastron multivesiculatum*, × 1650. Cycle shown by the dorsal vacuoles. Fig. 20, early diastole, frontal section; fig. 21, diastole, sagittal section; fig. 22, diastole, frontal section; figs. 23–24, middle of diastole, longitudinal sections; figs. 25–26, diastole, a comparison of the results of impregnation at 20°–24° C (fig. 25) and impregnation at 30°–32° C (fig. 26), frontal sections; fig. 27, late diastole, cross section; fig. 28, late diastole, cross section; fig. 29, resting stage, sagittal section; fig. 33, late diastole, sagittal section.

Figs. 30–32. *Metadinium medium*, × 800. Fig. 30, early diastole, cross-section; fig. 31, late diastole, parasagittal section; fig. 32, late diastole, cross-section. Photomicrographs by Mr. J. E. Gullberg.

Fig. 34. *Eudiplodinium maggii*, × 700. Posterior vacuole in early diastole. Frontal section. Compare with pl. 17, fig. 44.

Fig. 35. *Ostracodinium monolobum*, × 800. Anterior vacuoles in diastole, frontal section.

Fig. 36. *Eudiplodinium maggii*, × 250. Anterior vacuole in the resting stage, the posterior vacuole in early diastole, whole mount. Compare with pl. 17, fig. 47.

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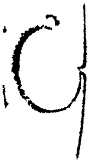

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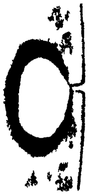

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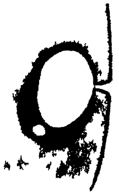

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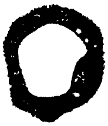

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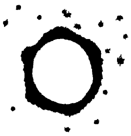

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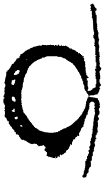

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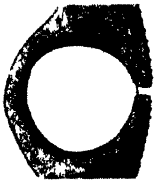

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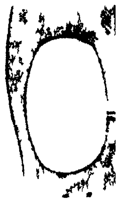

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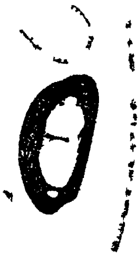

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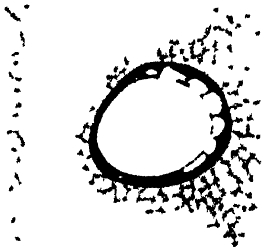

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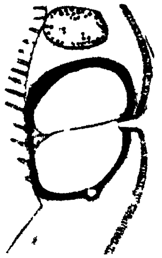

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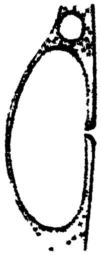

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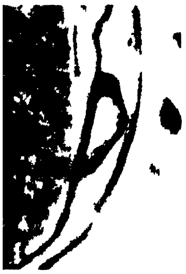

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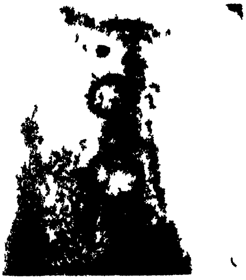

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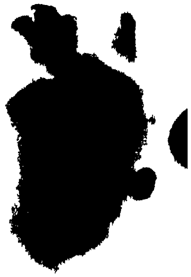

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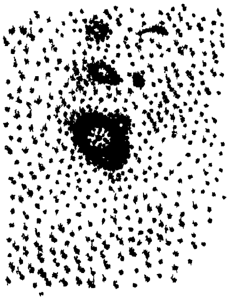
PLATE 18

Figs. 37–38, 40–43. ***Ostracodinium monolobum*****, × 660. Fig. 37, early diastole, frontal section; fig. 38, diastole, frontal section; fig. 40, late diastole, whole mount; fig. 41, diastole, sagittal section; fig. 42, resting stage, whole mount; fig. 43, late diastole, frontal section.**

Fig. 39. ***Epidinium caudatum*****, × 1500. Anterior vacuole in resting stage, cross-section.**

Figs. 44–49. ***Eudiplodinium maggii*****, × 1500. Fig. 44, posterior vacuole in diastole, frontal section; fig. 45, late diastole, frontal section; fig. 46, resting stage, frontal section; fig. 47, posterior vacuole at the beginning of systole; fig. 48, diastole, whole mount; fig. 49, late diastole, whole mount.**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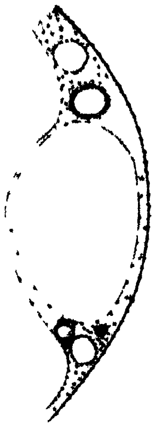
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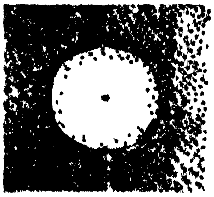
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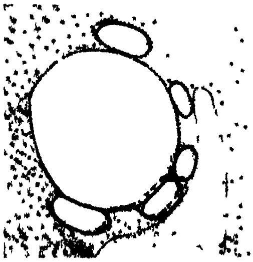
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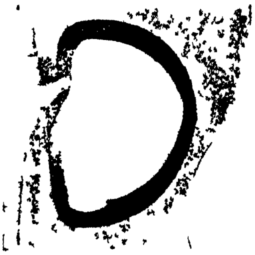
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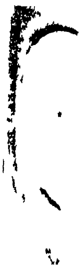
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THE MORPHOLOGY OF HAPTOPHRYA MICHIGANENSIS WOODHEAD, AN ASTOMATOUS CILIATE FROM THE INTESTINAL TRACT OF HEMIDACTYLIUM SCUTATUM (SCHLEGEL)

BY

MILDRED BUSH

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THE MORPHOLOGY OF HAPTOPHRYA MICHIGANENSIS WOODHEAD, AN ASTOMATOUS CILIATE FROM THE INTESTINAL TRACT OF HEMIDACTYLIUM SCUTATUM (SCHLEGEL)

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INTRODUCTION

Haptophrya michiganensis Woodhead is a large astomatous ciliate found in the intestinal tract of the four-toed salamander, *Hemidactylium scutatum* (Schlegel). The range of this salamander is from Massachusetts to Georgia and from Arkansas and Missouri to Michigan. It is a small form, the largest being only about five inches long, brown on the dorsal surface and white speckled with brown on the ventral surface. It creeps actively about in débris close to water but only the larval life is spent in the water (Blanchard, 1923).

The ciliate was discovered by Dr. F. N. Blanchard while he was working out the life-history of *Hemidactylium scutatum*. He (1923) found that about 70 per cent of the salamanders were infected with this parasite, and later he found one specimen of *Ambystoma jeffersonianum* which was also heavily infected. The ciliate was described and named by Dr. Arthur E. Woodhead (1928), of the University of Michigan.

ACKNOWLEDGMENTS

The morphology, particularly the fibrillar system, of *Haptophrya michiganensis*, which is here described, was worked out in the Zoological Laboratory of the University of California at Berkeley, at the suggestion of Dr. C. A. Kofoid, to whom I wish to express appreciation for assistance and helpful criticism. I am indebted also to Dr. Harold Kirby, Jr., for valuable suggestions in regard to the work, and to Dr. R. F. MacLennan for assistance with the osmic preparations.

SYSTEMATIC POSITION

Haptophrya michiganensis belongs to the family Haptophryidae Cépède (= Discophryidae Cépède, 1910). In his monograph on the astomatous Infusoria, Cépède (1910) included under the name Discophryidae four genera, *Lachmannella* Cépède, *Steinella* Cépède, *Discophrya* Stein, and *Haptophrya* (Stein, 1859) Cépède. Discussion has centered about the species *Discophrya planariarum* (Siebold) and *Haptophrya tritonis* Certes. Siebold (1845) gave the species the name of *Discophrya planariarum.* Stein (1859, p. 89) referred to the same species as *Opalina planariarum,* and also (1860) as *Discophrya planariarum.* In his ciliate classification (1867, p. 169) Stein used the generic name *Haptophrya* with *Discophrya* in parentheses. Maupas (1879) kept the name *Haptophrya* in describing *H. gigantea.* Kent (1882, pp. 568–571) retained the genus *Haptophrya* with three species, *H. gigantea, H. planariarum,* and *H. tritonis.* Bütschli (1889, p. 1717, pl. 65, fig. 2) used the generic name *Discophrya,* retained the species *H. gigantea* and *H. planariarum,* but rejected *H. tritonis* which he thought was probably a *Balantidium.*

Collin (1912) in his monograph on the Acinetaria pointed out that *Discophrya* had been used as a generic name by Lachmann in 1859 and suggested that *Sieboldiella* be used instead of *Discophrya* for the astomatous ciliate genus. Cépède (1923) changed the name of the family from Discophryidae to Haptophryidae and accepted the genus name *Sieboldiella.* He established subfamilies, Lachmannellinae, Steinellinae, and Haptophryinae, and placed *Sieboldiella* and *Steinella* in the subfamily Steinellinae. Bishop (1926) retained Cépède's classification of 1923 with the suggestion that a subfamily, Sieboldiellinae, be created for the genus *Sieboldiella,* since it differs in structure and mode of division from both *Steinella* and *Haptophrya.*

Cheissin (1930) considered these grounds insufficient for establishment of the subfamily and adoption of the generic name *Sieboldiella,* but accepted the family name, Haptophryidae, and kept the name *Haptophrya planariarum.* Since *Haptophrya* has priority over *Sieboldiella,* and since the classification given by Cheissin seems adequate for the family, this classification will be followed in this paper.

FAMILY *HAPTOPHRYIDAE* CÉPÈDE

1. LACHMANNELLA Cépède
   L. RECURVA Claparède et Lachmann

2. STEINELLA Cépède
   S. UNCINATA Schultze

3. HAPTOPHRYA Stein
   H. PLANARIARUM Siebold
   ?H. TRITONIS Certes
   H. GIGANTEA Maupas
   H. MICHIGANENSIS Woodhead

Haptophrya planariarum is found in the intestinal tract of a fresh-water triclad, *Planaria torva.*

Haptophrya tritonis was found by Raphael Blanchard in the intestinal tract of an Alpine *Triton* and was described briefly by Certes in 1879.

Haptophrya gigantea, the type species, described and named by Maupas in 1879, is found in *Discoglossus pictus,* a frog found in northern Africa. This species has also been found in *Rana esculenta.*

Haptophrya michiganensis is more closely related in size, form, structure, and habits to *H. gigantea* than to any of the other species.

MATERIAL AND METHODS

Some fixed parasites were obtained from the University of Michigan. Living salamanders were procured from the vicinity of Ann Arbor through the kindness of Dr. Woodhead and were kept for several months in cool, damp moss.

In order to obtain the parasites, the salamanders were killed and the intestinal tracts removed. If infection was at all heavy the ciliates could be seen through the intestinal wall, particularly just posterior to the stomach, in pink clumps which were sometimes so large that the intestine was distended to several times its normal diameter. In very heavy infections, some parasites could be found throughout the entire length of the small intestine and in the rectum. No parasites were found in the stomach.

For observation in living animals, the intestinal tract was put into tap water or into one-fourth normal saline solution in a watch glass and opened. Most of the ciliates are found attached by suckers to the intestinal wall, so close together that they give the appearance of a brush. A few very small ones of the bud type are found free in the intestinal contents. Sometimes large animals are found detached, curled up, and inclosed in a mucous sheath. The ciliates leave their places of attachment rather slowly but when detached move about freely, swimming by means of cilia which cover their entire surface, and by worm-like body undulations. If left for some time in the watch glass, many become attached to the bottom by their suckers.

A marked difference is to be noted between the forms in the anterior part of the intestine and those in its posterior portion and in the rectum. In the anterior part the animals are generally very long and few are to be seen in division stages. In the posterior portion the animals are generally short and are dividing rapidly, forming chains of three, four, or five segments. Occasionally a salamander is found in which the parasites are dividing rapidly throughout the entire length of the intestine and chains of three, four, or five animals are the rule. No periodicity of division was observed. The ciliates show a great variation in activity and in the granular content of the cytoplasm. The diameter of the animals remains about the same regardless of the length (fig. A, 1–3).

The parasites live in one-fourth normal saline solution for a week, the very short ones living the longest. Woodhead (1928) kept them alive in half-strength physiological salt solution for thirty days. After the first day, however, they become vacuolated and are unfit for study or for experimental purposes.

VITAL STAINING

Janus green B, Bismarck brown, and neutral red were used as vital stains. Janus green B in aqueous solution of about 1 to 10,000 parts very satisfactorily demonstrated mitochondria. Bismarck brown stained mitochondria but darkened the whole cell so that it was not particularly satisfactory for any definite structure.

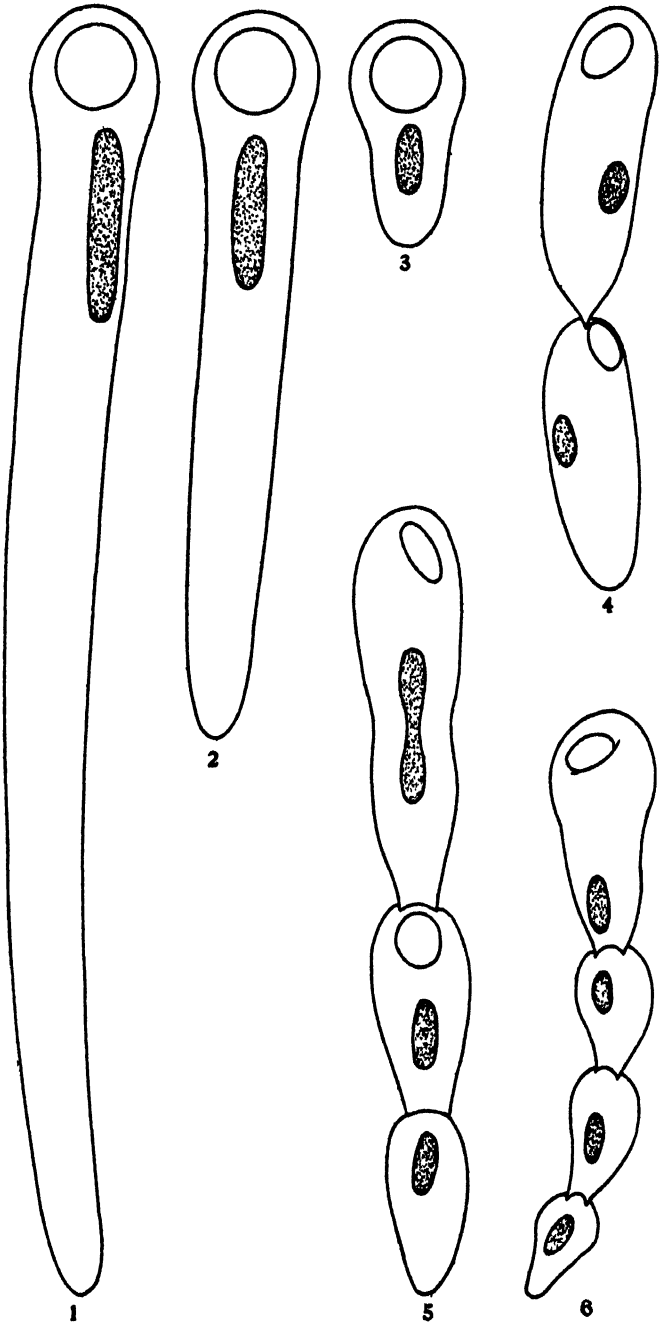

Fig. A. 1, 2, 3, camera lucida drawings showing various lengths of the ciliate. 4. Division showing primite and one satellite. 5. Division showing primite and two satellites. 6. Division showing primite and three satellites. All × 100.

Neutral red in weak aqueous solution was mixed on a coverslip with a drop of tap water containing the animals. The coverslip was then inverted over the cavity of a hanging-drop slide and sealed with vaseline. In using alcoholic neutral red, a coverslip was filmed and dried, after which a drop of water containing animals was put on the slip which was then inverted and sealed as before. In this way the animals could be observed conveniently. Staining required from fifteen minutes to an hour.

Some of the ciliates were treated with a mixture of neutral red and Janus green B in order to test differential staining.

Preparations for study could be kept a week or more by treating the stained material with a mixture of equal parts of glycerine and saturated solution of picrate of ammonium, and sealing the slides with paraffin, following the method of Mayer (1889). This process served to fix the color and preserve the animals.

FIXING AND STAINING

For whole mounts and for sections, the following fixatives were used: Schaudinn's, Zenker's, Bouin's, Champy's, Da Fano's cobalt-nitrate-formalin, and Zirkle's copper-dichromate mixture. Of these Schaudinn's, Zenker's, and Champy's gave the most satisfactory results.

For sections, pieces of intestine containing the parasites were fixed without disturbing the latter. This method proved to be more satisfactory than sectioning the free forms unless orientation of the animals was desirable. For free animals the capsule method of imbedding was followed. By using capsules cut in half longitudinally, the animals could be placed in any position desired. For study of the fibrillar structure the sucker was dissected out, stained, and destained under the microscope.

Various stains were used. Some good results were obtained with Delafield's hematoxylin, but, for general structure and for fibrils, Heidenhain's iron hematoxylin and osmic impregnation were the best. Counterstaining the hematoxylin preparations with eosin and light green made attractive slides but served no useful purpose. Mallory's triple connective tissue stain was used for fibrils.

Champy's, followed by osmic impregnation according to Kolatschev's method as described by Bowen (1928), proved to be excellent for study of the Golgi material and of the structure of the contractile canal. A section of intestine containing ciliates was fixed in Champy's at room temperature, washed for twenty-four hours in distilled water, and put into 2 per cent osmic acid. Eighteen to twenty days were required for satisfactory impregnation. After sectioning, the preparations were bleached in turpentine from twenty-four to forty-eight hours, twenty-four hours giving the more satisfactory results.

Da Fano's, followed by silver impregnation, according to the Yabroff method (1928), gave good results for the Golgi apparatus and also showed the structure of the accessory bodies of the neuromotorium. Impregnation required from seven to twelve days.

Klein's silverline method (1926) was used but with no great success. Gelei's silverline method (1931) was somewhat more satisfactory for this particular animal, since, in this method, the material does not require drying as in Klein's method (pl. 19, fig. 4). Noland's fixative and stain for flagella and cilia gave good results.

EXPERIMENTS

In coördination experiments the suckers were removed with very small knives, made from fine needles ground to an edge, under a dissecting microscope or under the low power of a binocular microscope.

MORPHOLOGY

OBSERVATIONS ON LIVING MATERIAL

SIZE AND FORM

Haptophrya michiganensis is an extremely large ciliate varying in length from 200 to 1580μ. The diameter at the anterior end is from 50 to 60μ and this diameter remains almost constant regardless of length. When the animal is swimming about, the body is slightly larger and somewhat flattened dorsoventrally at the anterior end, gradually tapering toward the posterior end which is about 15μ in diameter and is rounded, unless it has recently given off a bud (pl. 19, fig. 1). In that event the posterior end is almost square or has two projecting tips on the lateral margins, which mark the last two points of attachment to the posterior animal (fig. A, 4–6).

The most conspicuous parts of the ciliate are the sucker, the macronucleus, and the contractile canal (pl. 19, fig. 1). The large *sucker,* which is almost circular, is on the ventral side at the anterior end. When the animal is detached, the sucker is flattened and saucer-shaped, but when it is attached the sucker grasps from six to ten intestinal epithelial cells and is recessed in the form of a cup. The epithelial cells are drawn into the cavity forming a sort of papilla (pl. 20, figs. 17–18). It is obvious that the cells may be injured mechanically by the suction since the cilia are sometimes pulled away entirely.

The long, ellipsoidal *macronucleus* in the resting phase is located just behind the sucker, nearer the ventral than the dorsal side (pl. 20, fig. 14). It is sometimes very short but may be one-fifth as long as the body. Its length bears no constant ratio to the length of the animal (fig. A, 1–3). Its diameter does not vary greatly in different animals. The macronucleus can be seen easily unless the cytoplasmic granules are exceptionally abundant. Large smooth granules, each surrounded by a narrow halo, are visible imbedded in its surface. The membranous micronuclear sacs, which look like tiny bubbles, can be seen sometimes projecting slightly from the surface of the macronucleus.

The *contractile canal* appears as a tube along the dorsal surface, extending from the anterior end, dorsal to the sucker, to the posterior end, where it is attached to the ectoplasm. The ends of the canal are closed. Sometimes it is almost straight, sometimes convoluted until its length is greater than that of the animal. The canal opens to the outside by means of short, dorsally directed tubules located at rather regular intervals (pl. 21, fig. 20). The number of these tubules varies from five to fifteen or more, depending upon the length of the animal. The pores from these tubules can be seen as a series of openings along the mid-dorsal line in the living animal, and more distinctly in the stained material (pl. 19, figs. 2, 4). The first pore is usually dorsal to the posterior edge of the sucker, the last one about an equal distance from the posterior end.

The canal contracts rather irregularly, at intervals of from three to ten minutes. The contraction may begin at the anterior end and proceed posteriorly throughout the entire length of the canal, or it may begin at any point and stop at any point. It may begin on each side of a pore and proceed toward the pore. The contraction forces the contents of the canal out through the little tubes. The process can be followed better if the animals are put into water containing powdered carmine or India ink or into aqueous neutral red solution.

That the walls of the contractile canal are permanent and structurally intact is shown by the fact that, in sections, pieces of the canal become detached but retain their tubular form; in wounded animals, the canal may project beyond the broken surface.

In complete diastole, the contractile canal is about 10μ in diameter (pl. 21, figs. 23–24); in systole, it is about half that diameter (pl. 21, fig. 22). In diastole its walls are very thin and contain thin, flattened vacuoles (pl. 21, fig. 23). Not infrequently, thin cross-partitions divide the lumen into sinuses. As systole begins, the vacuoles expand and the walls of the canal contract until the vacuoles meet those opposite, thus closing the lumen of the canal (pl. 21, figs. 21–22). The vacuoles then discharge into the canal and the walls of the latter relax to the original diameter of diastole.

The animal is covered throughout with numerous, very fine *cilia*. Those back of the sucker are 5μ long and are arranged in close longitudinal rows. Those on the sucker and around its edge are 10μ long. Peculiar transverse rows of long cilia are found on the ventral side just posterior to the sucker. The cilia are all used in locomotion. There is no evidence that those on the sucker in any way assist adhesion.

In swimming, the cilia move in double spiral waves which cross, making a net-like pattern, beginning at the anterior part and traveling posteriorly. Sometimes the waves become almost longitudinal in the middle of the body and then form the pattern again at the back. This pattern is sometimes caught and retained in fixed animals. Particles of carmine which are drawn into the sucker area are borne as if in a whirlpool to its center, then are carried backward, sometimes being thrown rather violently aside by the long cilia just posterior to the sucker.

The pellicle is relatively thin but is resistant to pressure and to mechanical change. It is very permeable, since vital stains are taken quickly and a saline solution stronger than one-half normal will cause plasmolysis, distorting the animals greatly.

The *cytoplasm* is divided into two distinct regions, the ectoplasm and the endoplasm. The *ectoplasm* forms a thin, firm layer around the body, thickening somewhat at the posterior end, and forming a thick, disk-like pad which constitutes the greater part of the sucker at the ventral anterior end. The ectoplasm is somewhat vacuolated, especially in the posterior region and in the sucker pad.

The *endoplasm* is divided into two parts, an outer alveolar layer and an inner denser part. In individuals showing no sign of preparation for division, the inner part forms a long cone, large in the sucker region and tapering back to the posterior end. Large alveoli are found just back of the sucker. The macronucleus is included in the anterior part of the endoplasmic cone (pl. 19, fig. 1). This cone is broken down in dividing animals.

Cytoplasmic granules, which give the pink color to the parasite, are found in all parts of the endoplasm but are especially numerous between the ectoplasm and the endoplasm, around the contractile canal, and around the endoplasmic cone. These granules stream about in the protoplasm, being most active in the region of the contractile canal where the flow usually moves anteriorly.

Observations on Fixed Material

In animals stained with iron hematoxylin or impregnated by osmic acid, the cytoplasmic granules are so dark that the internal structure is obscured. Careful destaining and sectioning are necessary in order to work out the detailed structure of the organelles and to trace the entire fibrillar system.

ECTOPLASMIC STRUCTURES

The *ectoplasm,* which is separated from the endoplasm by a distinct line of demarcation, appears as a bluish, hyaline layer somewhat thickened in the posterior part and extremely thick in the sucker pad. Sections show that it consists of a thin pellicle, an alveolar layer, and thickened inner layer which is designated as the cortical plasma. The alveoli are especially noticeable in sections of the sucker, less so in the thickened posterior part and around the periphery of the animal. The contractile canal is evidently of ectoplasmic origin, since it appears as the peripheral ectoplasm, except that the vacuoles in its walls retain the stain (pl. 21, figs. 19–20).

Stained whole mounts show numerous, longitudinal rows of *cilia* with their basal granules, the latter deeply stained, running from the sucker somewhat spirally to the right when the animal is viewed from the ventral side. Longer cilia, with large basal granules which form a circle around the edge of the sucker, are crowded into indefinite rows. Posterior to the sucker, the long cilia, already noted in the living animal, have large basal granules and form branching rows which begin at a point on the animal's left and extend fan-shaped around to the right (pl. 20, fig. 11). Cilia on the sucker are longer than those on the remainder of the animal. Those on the sucker of a resting animal are arranged irregularly; those on one which is incompletely reorganized after division are in longitudinal rows.

Cross-sections show that the basal granules are large, rod-shaped, and almost as long as the peripheral ectoplasm is thick. Tangential sections show that the basal granules are connected by longitudinal fibers and by very delicate transverse commissures, which are observed with difficulty. A pattern of more or less regular squares results (pl. 19, fig. 2; *comm.*, fig. B, 2). Klein's and Gelei's silverline methods demonstrate this pattern in faint outline and

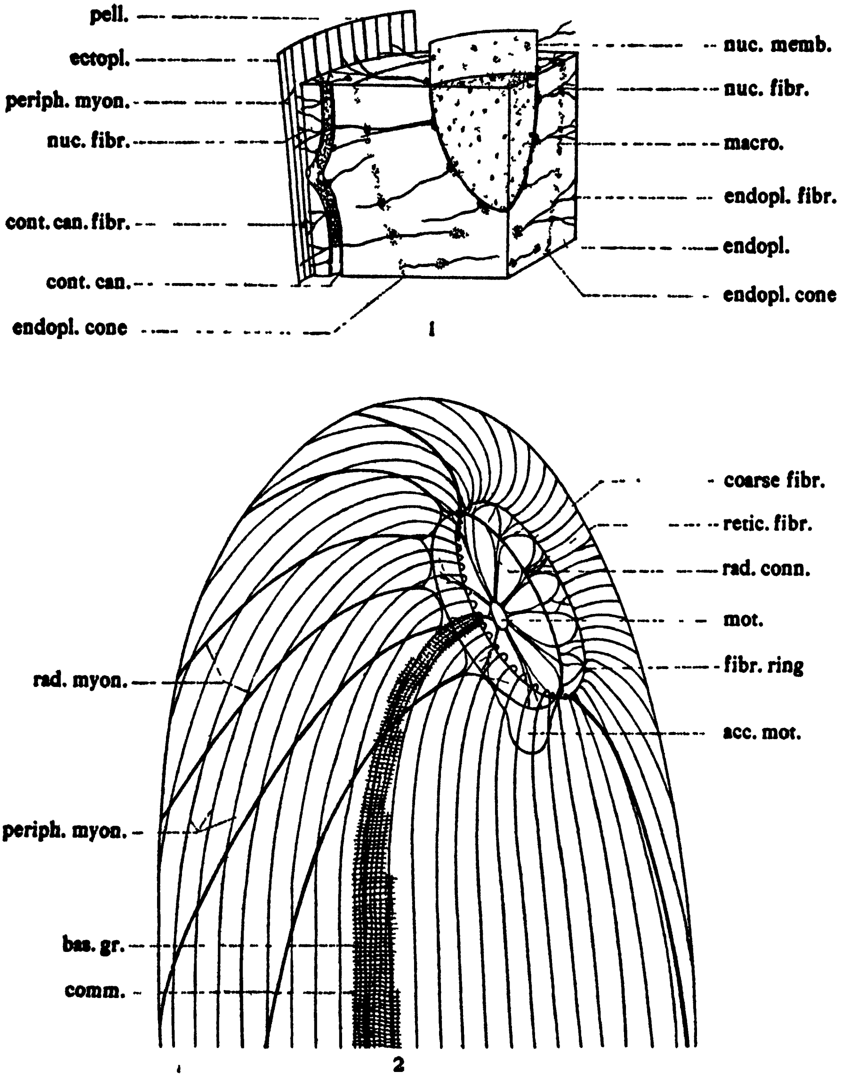

Fig. B. *Haptophrya michiganensis*. 1. Diagrammatic section showing one-fourth of the animal body in the region of the macronucleus, through the contractile canal. 2. Diagram of the anterior part of the neuromotor system: *acc. mot.*, accessory motorium; *bas. gr.*, basal granules; *comm.*, commissures; *coarse fibr.*, coarse fibrils; *cont. can.*, contractile canal; *cont. can. fibr.*, contractile canal fibrils; *ectopl.*, ectoplasm; *endopl.*, endoplasm; *endopl. cone*, endoplasmic cone; *endopl. fibr.*, endoplasmic fibrils; *fibr. ring*, fibrillar ring; *macro.*, macronucleus; *mot.*, motorium; *nuc. memb.*, nuclear membrane; *nuc. fibr.*, nuclear fibrils; *pell.*, pellicle; *periph. myon.*, peripheral myonemes; *rad. conn.*, radial connectives; *rad. myon.*, radial myonemes; *retic. fibr.*, reticulate fibrils.

stain deeply the granular lines around the contractile canal pores. Just beneath the cytoplasm, and adhering to it, are longitudinal myonemes which are stained with iron hematoxylin (pl. 19, figs. 3, 5). There are about forty-eight of these, almost equally spaced, around the middle of the animal, but there are fewer around the posterior part. Four ciliary rows to one myoneme is the average so that there are approximately 200 longtitudinal rows of cilia. The relation of the two is shown in sections (pl. 19, figs. 3, 5).

The *sucker* appears as a hyaline disk in the center of which is a dark-staining body surrounded, at a little distance, by a dark ring the diameter of which is about 30µ or two-thirds of the diameter of the disk (pl. 19, fig. 1; pl. 20, fig. 8). Fibrils radiate from the sucker region to the periphery (pl. 20, figs. 10, 13, 16; pl. 21, fig. 30; *rad. myon.*, fig. B, 2).

Staining brought out the conspicuous net-like appearance produced by the vacuoles in the *contractile canal* walls (pl. 21, figs. 19–20). The lines, which can be observed in living animals, are stained heavily by iron hematoxylin and are impregnated deeply by osmic acid and faintly by silver. The tubules leading from the lumen of the contractile canal through the ectoplasm to the surface are homogeneous, evidently of ectoplasmic origin, and they show no lines except around the pores (pl. 21, figs. 20, 22). During diastole these excretory tubules are guarded by temporary membranes which are broken when the canal contracts and forces its contents out.

The contractile canal is held in place by the excretory tubules and by fibrils which radiate from its surface outward to the ectoplasm and to the surrounding endoplasm. A vacuolated area is left around it, which probably facilitates its action (pl. 20, figs. 14–15).

ENDOPLASMIC STRUCTURES

As stated before, the *endoplasm* consists of two distinct parts, an outer alveolar part and an inner endoplasmic cone. The peripheral layer of the outer part, containing numerous cytoplasmic granules, consists of small alveoli while the inner part is highly vacuolated (pl. 20, figs. 14–15). The endoplasmic cone is very dense except in the anterior part adjoining the sucker, and around the macronucleus where the alveoli are extremely large. Granules are numerous around this cone (pl. 19, fig. 1). Fibrils radiate from the endoplasmic cone to the ectoplasm (pl. 20, fig. 15) and from the sucker obliquely across the large alveoli of the anterior part to the ectoplasm (pl. 20, figs. 10, 16).

Haptophrya michiganensis possesses one *macronucleus* and one or more micronuclei. The macronucleus is very large, varying from 100 to 175µ in length and from 20 to 25µ in width. It is peculiarly segmented, the segments being unequal and marked off by shallow constrictions which show plainly in stained material. The macronucleus, which stains readily with iron hematoxylin, consists of a spongy stroma supporting large chromatin granules. The granules can be observed in living animals. In stained preparations they contract somewhat but persistently retain the stain (pl. 21, fig. 28). Sections show that they are vacuolated. They vary in size from barely visible to 4μ, and are spherical, ovoid, club-shaped, or irregular in form. Some granules show division, particularly at the time of division of the macronucleus (pl. 21, fig. 26). The macronucleus is not impregnated by osmic acid or silver.

The *micronuclei,* of which there may be from one to six, or even as many as fourteen, lie in depressions in the macronucleus (pl. 21, fig. 28). They may be arranged in clusters or scattered at random over the surface. Each micronucleus is inclosed in a delicate membrane and is supported by fine fibrils which extend from the micronucleus to the membrane. They are ovoid or spindle-shaped and show minute granules which take the hematoxylin stain much as do the granules of the macronucleus (pl. 21, fig. 28).

A nuclear membrane incloses both the macronucleus and the micronuclei and this membrane is supported by fibrils which extend out to the ectoplasm (pl. 20, fig. 14; pl. 21, fig. 26).

Various types of *cytoplasmic* granules in this protozoan can be identified through selective staining and by a study of the size and form of the granules.

Janus green B used as an intra-vitam stain serves to distinguish granules varying in size and shape from barely visible spherical forms to the rod-like, pyriform, or irregular forms, some of which are 2μ in length. These granules are scattered at random throughout the endoplasm but are most numerous in the peripheral layers outside the endoplasmic cone. The rod-like and the small spherical granules are stained more deeply than the pyriform or irregular ones. These results are similar to those described by Richardson and Horning (1931) in a study of the protoplasmic structures in the binucleated opalinids. These workers conclude that the rod-like, deeply staining granules are the true mitochondria and that the pyriform and the irregular ones are "vegetative granules," a term used earlier by Horning (1925) to designate storage products which are synthesized at the surface of the mitochondria, given off into the cytoplasm, and absorbed. The close contact of some of the rods and the vegetative granules as described in the binucleated opalinids, was not observed in *Haptophrya michiganensis.*

Bismarck brown stained both the mitochondria and the granules as well as the cytoplasm very deeply and so was not satisfactory for differential purposes. All the cytoplasmic granules were stained by iron hematoxylin but only the mitochondria and the vegetative granules were stained by Janus green B. Excepting very small spherical particles, the mitochondria and the vegetative granules can be distinguished from the other granules by their peculiar forms. The granules stainable with Janus green B do not appear in fixed animals.

The *vacuome,* a term used to designate the sum total of the inclusions which are stained readily with neutral red, consists of very small granules and large, irregularly rounded granules about 1μ in diameter. The small granules are scattered throughout the endoplasm but the large granules collect around the contractile canal and are sometimes so numerous that they obscure it. They are in close contact with the outer surface of the canal but do not adhere to it nor enter the lumen of the canal. When the animal contorts, the granules are moved about and can be followed easily in the streaming protoplasm near the canal.

In the ciliates that were put into a mixture of equal parts of neutral red and Janus green B, the large granules around the contractile canal took up the neutral red quickly. The Janus green B was taken up slowly by the mitochondria and the vegetative granules but not at all by the large neutral red granules, indicating that the two are distinct elements.

Golgi apparatus appears in the form of uniform, small, spherical granules scattered throughout the ectoplasm and endoplasm. These small granules may be free, or surrounding, or partly surrounding a larger secretory particle (pl. 19, figs. 6–7). In sections the Golgi apparatus is most easily observed in the vacuolated endoplasm and around the contractile canal (pl. 19, fig. 7; pl. 20, fig. 17). Many granules are found in the small vacuoles of the sucker pad so that the latter appears almost black in osmic preparations (pl. 20, figs. 17–18).

The conclusion that these granules are Golgi elements is based on the criteria given by Bowen (1928), namely, (1) they are consistently impregnated by osmic methods, (2) by silver, and (3) the osmic- and the silver-impregnated granules resist destaining by ordinary methods. That the granules impregnated by osmic and by silver are the same is shown by the fact that they are similar in size, form, arrangement, and location. No other granules are deeply stained by the silver. In osmic preparations turpentine is a satisfactory bleaching agent. After several hours in this reagent, the secretory particles become gray while the Golgi elements remain black (pl. 19, fig. 6).

The membranous walls of the vacuoles formed in the contractile canal are darkened by the osmic impregnation, and to a lesser degree by the silver, giving the appearance of a network of lines over the surface of the entire canal, except over the discharging tubules whose walls are not vacuolated. The blackening was apparently due to a disintegration of Golgi material.

A neutral red preparation was made on a coverslip and inverted over the cavity of a hanging-drop slide containing osmic acid. The results were watched for three days. The large red granules along the contractile canal could be followed easily. They lost the red color but were not darkened by the osmic acid as the other granules were. These observations lead to the conclusion that in this protozoan the vacuome and the Golgi apparatus are two distinct elements, as was found by MacLennan (1932) to be true also in the Ophryoscolecidae.

The Golgi apparatus has been worked out by Nassonov (1925) in another astomatous ciliate, *Dogielella spherii* Poljansky. In this ciliate the Golgi material appears around the contractile vacuole as a fibrous, wreath-like circle which is completely filled when the vacuole is in the diastole phase. Hirschler (1924) suggests that the fibers are the result of over-impregnation due to warm fixation and that the Golgi material is in reality granular as is usual in the Protozoa.

King (1926) described large granules which are supposed to be Golgi material in *Hoplitophrya brasili* (Leger and Duboscq) Hentschel. These granules are large and pear shaped and consist of two materials, one deep-staining with iron hematoxylin after Champy's, the other light-staining. These granules are stained by neutral red also.

NEUROMOTOR SYSTEM

The term "neuromotor apparatus" is used here to designate an integrated system of fibrils which center in a mass located in the sucker. Because of the arrangement of the fibrils and of experimental results, it seems probable that this system is concerned with the coordination of the movements of the ciliate.

The *neuromotor apparatus* consists of six parts: (1) a central motorium with accessory bodies; (2) a fibrillar ring homologous to the esophageal ring of other forms; (3) radial myonemes from the internal surface of the sucker to the ectoplasm; (4) peripheral myonemes; (5) fibrils connecting the basal granules; (6) supporting fibrils of the nuclei.

In order to coördinate all parts of the system, it is necessary to begin with a description of the *fibrillar ring*. This ring appears at first glance to be merely a dark circle in the thickest part of the sucker. Detailed study, however, discloses that it consists of fibrils arranged in a band-like ring with the width of the band equal to the thickness of the sucker pad. Coarse fibers form loops in the band and these support a reticulated arrangement of more delicate fibers except in the ventral part where no coarse fibers are in evidence and where the finer fibers form a much smaller-meshed network (*fibr. ring,* fig. B, 2; pl. 20, fig. 8). In osmic fixation, which makes the animals extremely brittle, the coarse fibrillar structure is sometimes found detached from the rest of the system.

In the center of the fibrillar ring is a mass extending inward from the interior surface of the sucker and varying in size from quite small to about one-fourth of the diameter of the fibrillar ring. It is attached to this ring at its inner edge by radial connectives (*rad. conn.*, fig. B, 2; pl. 20, figs. 8, 9, 12). This mass will be designated as the *motorium*, a center for coördinating the motor parts of the animal. The reasons for this are as follows: (1) it is connected, directly or indirectly, with all parts of the fibrillar system; (2) it is near the anterior end of the ciliate; (3) if, with this mass, the sucker is removed, the animal loses its power of worm-like forward movement even though the cilia continue to beat; and (4) a toxic substance acts first upon the anterior part, particularly the sucker, whereupon the animal ceases its forward movement. The motorium can be seen in some living specimens as a transparent mass, outlined when the animals are put into aqueous neutral red solution. It stains deeply with iron hematoxylin and is easily detached.

Accessory bodies which, the staining seems to indicate, are similar in structure to the motorium, may be found suspended from various points on the inner edge of the ring, but the largest and most often seen is suspended from the reticulated threads of the ventral part of the ring (pl. 21, figs. 29–32). This accessory body appears in various forms and may be connected with the motorium by a mass of material similar in structure. Fragments of this body are given off, move backward, divide, and, certainly in some individuals, disintegrate. Staining shows that the accessory body and the fragments are alike in structure and that they are made up of two materials. Heidenhain's iron hematoxylin stains some parts much more deeply than others (pl. 21, figs. 29–32). With silver, the fragments become yellow in the outer part and pink in the inner (pl. 21, fig. 25). It is noticeable that neither the accessory bodies nor the fragments are evident in dividing forms. The neuromotorium can be seen in some of the anterior animals of dividing catenular chains. A large, ventral, accessory body can occasionally be observed in living animals.

Long, very coarse, *radial myonemes* are given off from the sucker at the inner edge of the fibrillar ring, both dorsally and laterally. They extend radially to the ectoplasm, dividing into fine fibrils at their outer ends (pl. 20, figs. 10, 13, 16; pl. 21, figs. 29, 31). At least some of the fine fibrils connect with peripheral myonemes. Similar fibrils are given off from the sucker outside of the ring (pl. 20, figs. 9, 10, 13). This arrangement of fibers leaves large alveolar spaces unobstructed for the macronucleus and the accessory bodies.

The *peripheral myonemes* arise at about equal spaces from the external edge of the fibrillar ring and continue to the posterior end, sometimes branching anteriorly, and anastomosing posteriorly (pl. 19, fig. 5; *periph. myon.*, fig. B, 2). These fibrils adhere to the cortical plasma and are closely associated' with the basal granules (pl. 19, figs. 3, 5).

Delicate commissures connect the rod-shaped *basal granules* at their inner ends, making a close network over the entire body (pl. 19, fig. 2; *bas. gr., comm.*, fig. B, 2).

Fibrils which support the nuclear membrane originate in blobs of cytoplasm on the membrane and extend to the ectoplasm, branching at their outer ends. Clumps of granules may be in evidence where these fibers extend through the outer margin of the endoplasmic cone (pl. 20, fig. 14; *nuc. fibr.*, fig. B, 1). Fibrils also arise in the cytoplasmic cone and extend out to the ectoplasm (pl. 20, fig. 15; *endopl. fibr.*, fig. B, 1).

Mallory's triple connective tissue stain, usually specific for neural fibers, did not given the distinctive results in this ciliate that were described by Sharp (1914) in *Diplodinium*. The fibers, however, were stained somewhat more deeply than the surrounding cytoplasm.

REPRODUCTION BY CATENULAR BUDDING

Asexual reproduction by transverse division is the usual method of multiplication in *Haptophrya michiganensis*. The animal divides into two; then the posterior animal divides before it is completely reorganized and before the anterior animal has divided again (fig. A, 5). By a repetition of this division a chain of five, six, or more animals is produced. Of these, the anterior one is the longest and is designated as the primite, the others as satellites (Maupas, 1879). When there are three animals in a chain, they are graded in length, the posterior one being the shortest (fig. A, 5, 6). The buds produced by division are much smaller than the original animal.

Several changes take place in an animal preparing for division. The number of micronuclei increases and these distribute themselves near the ends of the macronucleus so that when the latter divides each half will be accompanied by several micronuclei. In one specimen, eight were seen on the anterior half of a dividing macronucleus and six on the posterior half (pl. 21, fig. 26). The division stages could not be followed, but numerous elongated and thickened micronuclei were found which showed both fine granules and blobs of deep-staining material irregularly arranged (pl. 21, fig. 27). The dense endoplasmic cone breaks down, allowing the macronucleus, with the micronuclei, to move posteriorly. When about halfway back, the macronucleus constricts into two equal, or almost equal, parts (pl. 21, fig. 26). Occasionally a small piece of a daughter macronucleus will be constricted off at the level of division, but this is not the rule. Woodhead (1928) apparently regarded this as an indication of macronuclear degeneration.

A clear band is formed in the middle of the body at right angles to the dividing macronucleus and the body wall constricts until the two animals are eventually separated. The last two points of attachment are two tips on the lateral margins of the posterior part of the anterior animal, which are attached one on each side of the newly formed sucker of the posterior animal. The two animals do not become independent until the sucker on the posterior is completely formed. The new suckers are not in a direct line with the original sucker, but at an angle of about 30 degrees (fig. A, 4).

The contractile canal is pinched in two, the anterior part remaining attached to the ectoplasm of the first animal, the posterior part forming a blind sac in the anterior end of the second animal.

As previously stated, neither the accessory bodies of the motorium nor the fragments are seen in dividing forms, though the motorium can sometimes be seen in the center of the sucker of the anterior animal. Subsequent history of the dividing animals could not be followed.

Neither conjugation nor encystment was observed.

EXPERIMENTS

SUSCEPTIBILITY GRADIENT

During the preparation of the material used, it was noticed that various substances, such as strong neutral red solution and turpentine, caused a cessation of movement of cilia at the anterior end first. This, together with the size, structure, and method of reproduction by catenular budding, suggested that this protozoan would be good material with which to test the susceptibility gradient theory (Child, 1914). KCN in 0.1 per cent solution and 35 per cent alcohol were used in addition to the neutral red and turpentine.

Strong neutral red solution caused a cessation of movement of the cilia at the anterior end first, gradually becoming effective backward along the cell; the cilia at the posterior end were the last to beat. Turpentine, used as a lipoid test, gave results similar to those produced by the strong neutral red, but, in addition, as it became effective it also removed the cilia antero-posteriorly. The cilia, many of them with their basal granules, moved about by Brownian movement, making it easy to follow the effects of the poison.

The cyanide and alcohol gave results similar to those described above. The ciliates were stimulated at first and swam about rapidly, then began to die, the forward-directed movement ceasing when the poison affected the sucker region.

In dividing specimens consisting of the primite and one satellite, the effect of the toxic substance was first noticed in the sucker of the primite, then in the sucker of the satellite. Inactivity proceeded gradually posteriorly from each sucker until all cilia ceased to vibrate, those at the extreme posterior end being the last to cease movement.

In chains consisting of primite and two satellites, inactivity began at the sucker of the primite and continued backward. When about a fourth of the primite had become inactive, the sucker of the first satellite became inactive, then the sucker of the second satellite. The toxic effect proceeded backward from these three points. These results varied considerably with the stage of division. If the new suckers were well advanced in organization and the buds were almost ready to separate, the sucker region seemed to be more susceptible than in the earlier stages.

These results indicate that the sucker regions have a high rate of metabolism and that the rate is higher when the sucker is well organized than when division has just begun.

TRANSMISSION

No cysts of *Haptophrya michiganensis* have been found and the method of transmission is as yet unknown. In an effort to find the method of transmission of the parasite and to learn whether either *Batrachoseps attenuatus,* the California salamander most like *Hemidactylium scutatum* in appearance and habits, or the larval form of *Triturus torosus* is susceptible to the infection, the following experiments were performed. (1) Parasites in all stages found in the intestine of *Hemidactylium* were fed to six Batrachoseps. (2) Parasites in all stages found were injected into the rectum of each of six Batrachoseps. (3) Fifteen Hemidactylium and twelve Batrachoseps were put into the same jar and left for twelve weeks. (4) Parasites in all stages found were put into tap water with a dozen larvae of *Triturus torosus.*

The Batrachoseps and the Triturus larvae were killed at intervals of from one to two weeks over a period of three months. No ciliates were found in the intestine of the Batrachoseps to which the parasites were fed, nor in those which received the injected parasites. Of the twelve associated in the jar with *Hemidactylium,* two contained a few dead parasites each after six and eight weeks, respectively. The dead parasites may have been the forms surrounded by a mucous sheath, described earlier in this paper, which occur in the intestine of *Hemidactylium,* and which may have passed out of the intestine and been taken up by the Batrachoseps. No ciliates were found in the intestines of the larvae of Triturus.

SUMMARY

Haptophrya michiganensis, an astomatous ciliate, is particularly interesting because of its extremely large size, its structure, and its habit of living attached by a sucker to the epithelial lining of the intestine of the four-toed salamander, *Hemidactylium scutatum.* Much of its structure can be observed in the living form. The sucker, macronucleus, contractile canal, and endoplasmic cone, are specially noticeable. The cytostome has probably been lost as a result of parasitism. The contractile canal of *Haptophrya michiganensis,* like that of *Haptophrya gigantea,* consists of a long tube with numerous, dorsally directed excretory pores. It has developed apparently from the fusion of many contractile vacuoles, each with its own excretory pore. The ciliate is entirely covered by about two hundred rows of cilia and moves about by ciliary action and by worm-like undulations of the body.

The sucker consists mainly of a thick, disk-like ectoplasmic formation. The macronucleus, which contains large chromatin particles, and the micronucleus are enclosed in a common membrane. The contractile canal is a permanent structure and appears as a tube enclosed by a network of lines—an appearance due to the walls of the vacuoles formed within the tube.

Haptophrya michiganensis has an integrated neuromotor system consisting of the following parts: (1) a fibrillar ring homologous with the esophageal ring of stomatous ciliates; (2) a motorium in the center of the fibrillar ring and connected with it by radial connectives—associated with the motorium are accessory bodies of varying size and shape; (3) numerous myonemes radiating from the interior surface of the sucker to the opposite walls, posteriorly and laterally; (4) equally spaced peripheral myonemes arising from the exterior edge of the fibrillar ring, adhering to the cortical plasma of the ectoplasm and extending to the posterior end of the animal; (5) commissures connecting the basal granules, forming a close network over the entire body; (6) supporting fibers from the nuclear membrane, the endoplasmic cone, and the contractile canal to the peripheral ectoplasm.

Differential staining was used to identify mitochondria, vegetative granules, neutral red granules (vacuome), and Golgi apparatus. The three types of granules were found to be distinctly separate in this ciliate.

Reproduction by catenular budding is described, but no cysts were found nor was conjugation observed.

Exposing the animals to various toxic substances showed that they were most susceptible to poisons in the sucker region, which is probably the region of greatest metabolic activity. This susceptibility develops progressively in the satellites of the catenular chains.

Attempts to transmit the parasite to *Batrachoseps attenuatus* and to the larvae of *Triturus torosus* were unsuccessful.

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida. Abbreviations for methods of preparation: Ch., Champy's fluid; D., Da Fano's fluid; G., Gelei's silverline; H., Heidenhain's iron hematoxylin; O., osmic impregnation; S., Schaudinn's fluid; Y., Yabroff's silver impregnation; Z., Zenker's fluid.

PLATE 19

Fig. 1. Ventral view of *Haptophrya michiganensis*, showing sucker, macronucleus, and contractile canal. No staining. × 350.

Fig. 2. Section showing basal granules with longitudinal fibrils and transverse commissures, and a pore of an excretory tubule. S. H. × 1000.

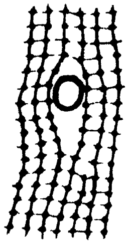
Fig. 3. Cross-section of ectoplasm showing cilia, basal granules, pellicle, alveolar layer, cortical plasma, peripheral myonemes. S. H. × 1000.

Fig. 4. Section showing basal granules with connectives and excretory pore. G. × 1000.

Fig. 5. Section of cytoplasm showing basal granules and peripheral myonemes. S. H. × 1000.

Fig. 6. Golgi apparatus. Ch. O. × 1000.

Fig. 7. Golgi apparatus around contractile canal. D. Y. × 1000.



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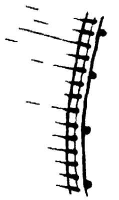

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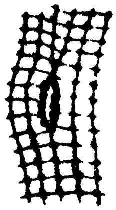

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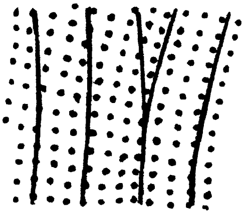

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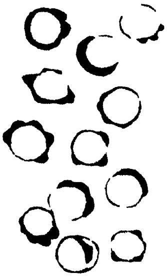

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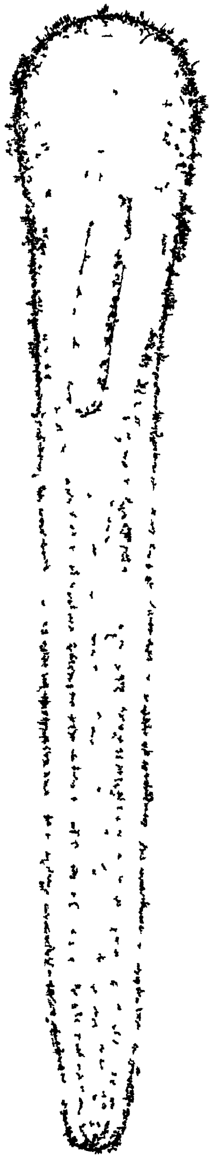

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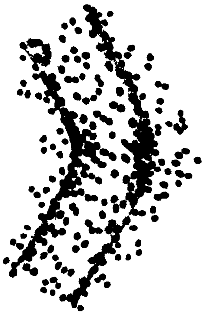

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PLATE 20

Fig. 8. Anterior ventral end of *Haptophrya michiganensis* showing arrangement of fibrils in sucker. S. H. × 480.

Fig. 9. Sagittal section through the anterior end showing motorium, macronucleus, and fibrils. Z. H. × 480.

Fig. 10. Sagittal section through sucker showing the ventral accessory body of motorium. Z. H. × 480.

Fig. 11. Basal granules of sucker region and beginning of longitudinal ciliary rows. S. H. × 480.

Fig. 12. Tangential section through inner part of sucker showing motorium with radial connectives. Z. H. × 480.

Fig. 13. Tangential section through sucker, following that in figure 12, showing radial myonemes. Z. H. × 480.

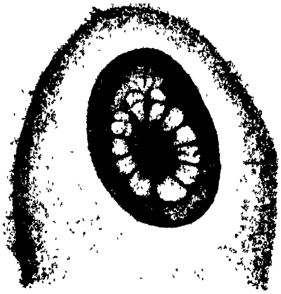
Fig. 14. Horizontal section of body through macronucleus, showing contractile canal and cytoplasmic cone. Z. H. × 480.

Fig. 15. Horizontal section through body behind macronucleus, showing cross-section of endoplasmic cone. Z. H. × 480.

Fig. 16. Transverse longitudinal section through sucker showing motorium, macronucleus, and radial myonemes. Z. H. × 480.

Fig. 17. Sagittal section through sucker showing ciliate grasping epithelial cells of intestine. Ch. O. × 480.

Fig. 18. Tangential section through sucker showing sucker grasping epithelial cells. Ch. O. × 480.



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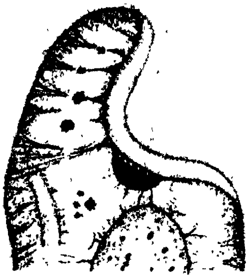

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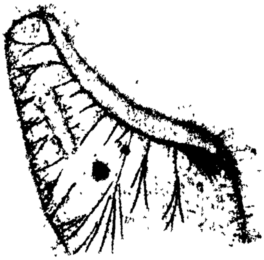

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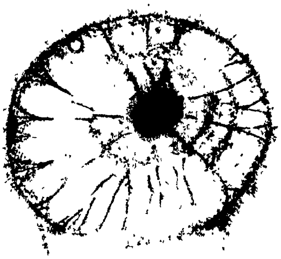

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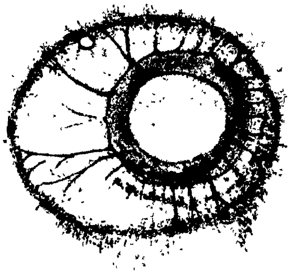

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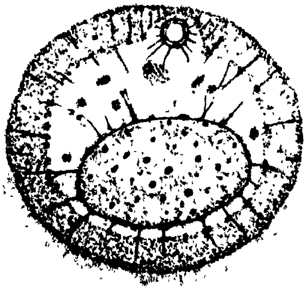

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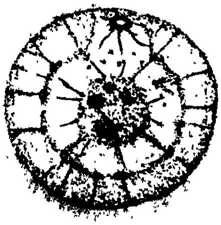

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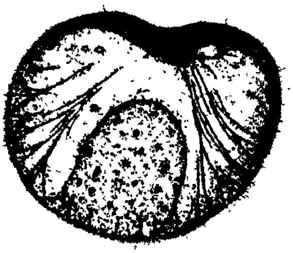

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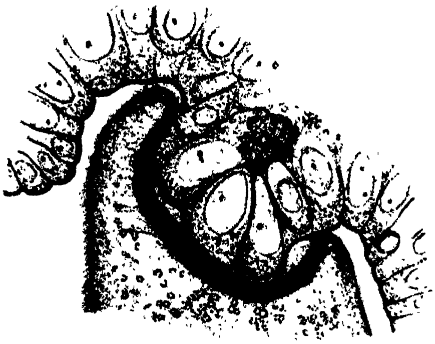

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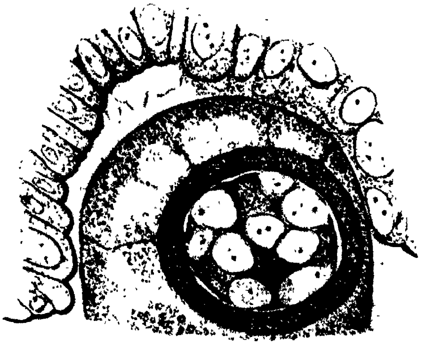

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PLATE 21

Figs. 19–20. Sections of the contractile canal showing net-like pattern made by vacuole walls. Figure 20 shows excretory tubule through ectoplasm. S. H. × 800.

Fig. 21. Longitudinal section of contractile canal showing vacuole formation. S. H. × 800.

Fig. 22. Longitudinal section of contractile canal at excretory pore in systole; vacuoles closing lumen. S. H. × 800.

Fig. 23. Longitudinal section of contractile canal in diastole, showing structure of walls of canal. Ch. O. × 800.

Fig. 24. Cross-section of contractile canal in diastole. Ch. O. × 800.

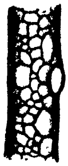
Fig. 25. Fragment of accessory body of motorium showing structure. D. Y. × 1000.

Fig. 26. Macronucleus in division; micronuclei on surface. S. H. × 480.

Fig. 27. Micronucleus enlarged, presumably preparing to divide. S. H. × 1000.

Fig. 28. Macronucleus with micronuclei imbedded in its surface, showing structure of both nuclei. S. H. × 1000.

Figs. 29–32. Anterior end of ciliate showing fibrillar ring, radial myonemes, motorium, accessory bodies, and fragments of accessory bodies. Z. H. × 480.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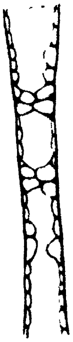
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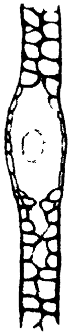
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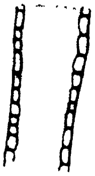
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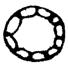
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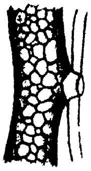
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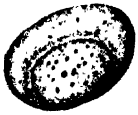
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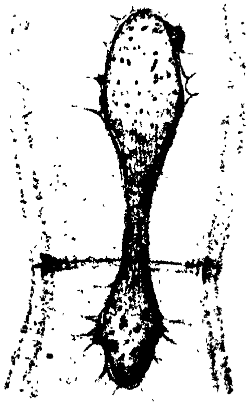
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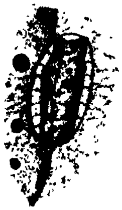
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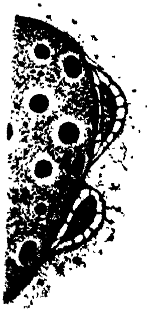
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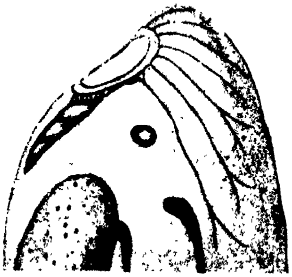
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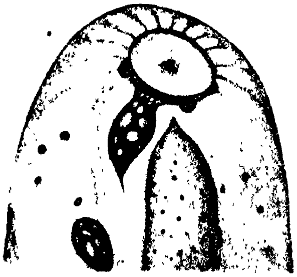
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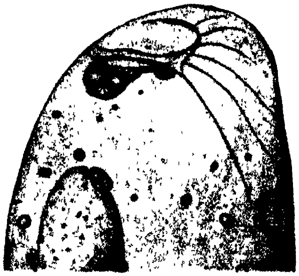
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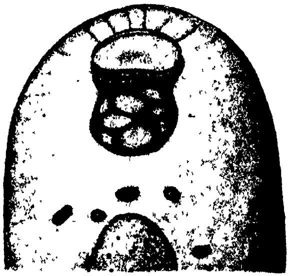
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THE ORGANIZER IN TRITURUS TOROSUS (RATHKE) AND ITS RÔLE IN THE DEVELOPMENT OF THE MEDULLARY PLATE

BY

A. MANDEL SCHECHTMAN

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THE ORGANIZER IN TRITURUS TOROSUS (RATHKE) AND ITS RÔLE IN THE DEVELOPMENT OF THE MEDULLARY PLATE

BY

A. MANDEL SCHECHTMAN

INTRODUCTION

THE FACTORS CONCERNED in the formation of the medullary plate in urodeles have been the subject of several recent theories. The most fundamental contribution was that of Spemann and H. Mangold (1924) who found that the dorsal blastoporal lip of the early gastrula can induce a neural tube in presumptive epidermis. A short time later Marx (1925) showed that the archenteric roof functions as an organizer, and he suggested that the formative stimulus which changes the development of the ectoderm in the direction of medullary material emanates from the archenteric roof. Holtfreter (1933*b*) considers a subjacent archenteric roof absolutely necessary to the formation of the medullary plate; no self-differentiative factors whatsoever are involved: "Die Enstehung der Medullarplate ist also nicht doppelt gesichert, sondern der mesodermalen Unterlagerung fällt die volle Verantwortung dafür zu, wie sich die indifferente Ektodermschicht formal und histologisch gestaltet" (p. 411). Goerttler (1927) described experiments which led him to the conclusion that differentiation of the neural anlage depends upon characteristic movements which occur in this material. This has been termed the theory of dynamic determination. Holtfreter (1933*a*) could not verify Goerttler's results and denies any causal relationship between movements of materials as such and medullary differentiation.

The formation of the medullary plate may also be explained as completely dependent upon self-inherent factors. Whether or not such factors are to be identified with those postulated by Goerttler, is immaterial. This may be referred to as the theory of self-differentiation, and the results of Goerttler's experiments upon axolotl and *Pleurodeles* (1926) lend it support. Erdmann (1931) holds that, in *Amblystoma,* pure presumptive medullary material of the blastula, isolated long before it becomes lined with archenteric roof, is capable of developing into medullary plate.

Lehmann (1928) conceives of the determination of the neural plate as a double process, involving both self-differentiation and induction by the archenteric roof. The medullary anlage at the beginning of gastrulation is considered a center of labile determination, which gradually fades out at the periphery into indifferent material. The archenteric roof in some way supplements the partial determination already present in the medullary anlage.

The aim of the present series of experiments was twofold. It seemed desirable, in the first place, to ascertain the extent to which certain results obtained with European Amphibia, notably *Triton*, are applicable to *Triturus torosus*, the most abundant urodele on the Pacific Coast of North America. Secondly, it seemed possible that a study of the degree of differentiation occurring in explants grown *in vitro* with and without the organizer might throw some light upon certain factors involved in the emergence of the medullary plate in *Triturus*. The problem was undertaken at the suggestion of Professor J. Frank Daniel, and was carried out during the spawning season of 1933.

MATERIALS AND METHODS

ALL EXPERIMENTS were made exclusively upon *Triturus torosus*. The fact that eggs of this salamander vary greatly in degree of pigmentation increases their value in transplantation experiments, for a darkly pigmented egg may be used as donor, and a relatively lightly pigmented specimen may serve as host.

The operations were performed by means of the hair loop and steel needle-knife in wax-bottomed Stender dishes. Eggs selected for operation were washed seven to ten times in sterile water and were kept during and after the operation in Stender dishes half-filled with sterile Holtfreter's solution (Holtfreter, 1931). Hair loops and needles were sterilized in 70 per cent alcohol and rinsed several times in sterile water just before use.

The operations performed involved the removal of rectangular pieces of tissue from embryos in various stages of development. In the transplantation experiments the early gastrula served as host. The implant was inserted by way of a slit made in the chorion *(Dotterhäutchen)* and the roof of the gastrula. As gastrulation proceeds the implant usually comes to lie between the ectoderm and the yolk mass of the host. Retention of the chorion was found to be of importance both in reducing the percentage of infections and in permitting normal gastrulation. In the explantation experiments the bits of tissue were maintained upon beeswax in Holtfreter's solution.

A total of 889 operations was performed. A representative group composed both of specimens showing induction and those in which no induction was evident was selected for sectioning. Embryos were fixed in Bouin's fluid, imbedded in paraffin (M.P. 55°–58° C) containing a small amount of beeswax, cut 10μ thick, and stained with dilute picro-indigo-carmine followed by Delafield's hematoxylin. In sections lightly stained the natural brown pigment of the egg is easily seen, and thus identification of the implant is facilitated.

TOPOGRAPHICAL MAP OF THE EARLY GASTRULA

THE TOPOGRAPHICAL MAP indicating the presumptive organ anlagen of the early gastrula in *Triton* (Vogt, 1929) has provided a more accurate basis than hitherto available for experiments in which the reactions of the various anlagen are tested under different conditions. While vital-staining experiments upon *Triturus torosus* have shown that the boundary between presumptive epidermal and medullary materials occurs, as in *Triton*, in the region of the animal pole, or approximately opposite the early blastopore (Schechtman, 1932), the boundaries of the various other anlagen have not yet been ascertained. Since, however, the validity of the experiments herein described depends in part upon a knowledge of the topographical relationships of certain presumptive anlagen, preliminary vital-staining experiments were carried out. The following experiment is illustrative of the results obtained.

In vital-staining experiment number 39, three series of alternating red and blue marks were placed on the early gastrula, one side of which is shown in plate 22, figure 1. These marks were observed during subsequent development until the medullary plate appeared (pl. 22, fig. 2). Since marks *18–15* appeared on the anterior portion of the medullary folds and extended into the adjacent epidermis, the boundary between presumptive medullary and epidermal materials of the early gastrula may be drawn as a line extending from mark *18* through and below mark *15*. The location of this boundary shows some variation even among some eggs from the same clutch.

Marks *4–1* were carried toward the blastopore during gastrulation, and in the neurula were observed in the epidermis (pl. 22, fig. 2). Only mark *1* encroaches upon the medullary fold. We may therefore conclude that in the early gastrula the corresponding series of marks occurred near the lower end of the presumptive epidermal material and that the region between marks *4–1* and *18–15* is presumptive epidermis. This was further shown by actually placing marks within this territory.

Marks *12–14* stretched toward the blastopore during gastrulation and came to occupy a portion of the left side of the medullary plate, extending only a very short distance into the blastopore. We may therefore represent the lower boundary of the presumptive medullary material as a line which would pass through the lower edges of marks *12–14*.

The information thus derived forms a sufficient basis for the present experiments, allowing us with a certain degree of accuracy (see p. 283) to extirpate areas of known prospective significance and to observe their reactions under a variety of experimental conditions.

LOCALIZATION OF THE ORGANIZER

Transplants from the Early Gastrula Stage

Transplants were obtained from five regions of the early gastrula: (1) presumptive epidermis, (2) presumptive medullary plate, (3) dorsal lip of blastopore, (4) lateral lips of blastopore, and (5) ventral lip of blastopore. In about 75 per cent of the host embryos receiving dorsal lip implants, the epidermis lying above the implant develops into a secondary medullary plate. The cells elongate greatly in a direction roughly perpendicular to the surface of the embryo. The nuclei become elliptical, their major axes tending to lie parallel to one another. The adjacent epidermis is lifted into folds. Externally the secondary medullary plate appears as a definitely delimited, somewhat sunken, and darkly pigmented area of variable form. During subsequent development the secondary medullary folds approach each other and fuse, thus producing a neural tube.

Embryo number 833 presents a typical example (pl. 22, figs. 3, 4, 5). The implant was composed of the right half of the dorsal blastoporal lip. Both the primary and the secondary medullary plates were already well developed when the egg was observed for the first time after the operation (fig. 3). The secondary plate was bifurcate anteriorly, with well developed folds, and darker in color than the rest of the egg. At the next observation the folds had closed (fig. 4). The previously observed anterior bifurcation of the secondary neural structure was not noticeable; a stubby, tail-like extension was seen near the posterior end. Sections of the embryo revealed two neural tubes of about equal size (pl. 22, fig. 5, *nt.* and *nt*^{2}.). The implanted right half of the dorsal lip had differentiated into chorda and somite material.

The present specimen also affords an opportunity to describe certain relationships observed throughout the experiment between the morphology of the secondary neural tube and the configuration of the organizing material. In a larger part of the present specimen the secondary neural tube has the typical form, the walls being thickened while the roof and floor are relatively thin (pl. 22, fig. 6 *nt*^{2}.). This condition gives way to one in which the basal portion of the tube is composed of cells more elongated than those forming the roof. The transition between the above-mentioned two types of neural tubes is shown in figure 5.

Correlated with this changing morphology in different regions of the tube are certain configurations of the implanted chorda and somite materials. The typically formed portion of the tube occurs as long as the chorda remains in contact with it, dividing the somite material into two unequal masses (fig. 6). The transitional condition shown in figure 5 occurs just before the chorda becomes separated from the secondary neural structure by somite material; thereafter the anomalous type of neural tube shown in plate 22, figure 7 is present. The relationships observed are in all essentials similar to those which have been recorded in the common English frog by Jenkinson (1915), and in *Triton* by Lehmann (1926, 1928) and by others, indicating the formative influence of the chorda and somites in the morphogenesis of the neural tube.

Implantation of the lateral blastoporal lips is also followed by the appearance of a secondary medullary plate. Ventral lip implants, however, in many specimens produced no external evidence of induction. Occasionally, structures which superficially resembled small neural folds were observed. The sections of such specimens showed them to be pseudo-medullary structures, entirely lacking in the histological characteristics of neural material.

Presumptive medullary or epidermal materials gave no evidence of inductive capacities. Several doubtful structures were shown to be pseudo-medullary; the superficial resemblance to a neural anlage was probably caused by the peculiar configuration of the underlying implanted material.

TRANSPLANTS FROM THE MIDDLE GASTRULA STAGE

Embryos designated as mid-gastrulae include those having blastopores in the form of a *U* and those with large circular blastopores. Transplants from the following regions were put into the blastocoele, early gastrulae being used as hosts: (1) presumptive epidermis, (2) presumptive medullary plate, (3) dorsal lip of the blastopore, (4) lateral lip of the blastopore, (5) ventral lip of the blastopore, and (6) archenteric roof. In this series of experiments only the dorsal and lateral lips and the archenteric roof implants induced secondary medullary plates.

Implants of archenteric roof material were obtained by extirpating a wide, mid-dorsal strip of the embryo, extending from the blastopore to the opposite pole. The strip thus removed consists of two layers, of which the internal is the archenteric roof. It is separated from the external by means of the hair loop and needle, cut up into pieces of desirable size, and implanted.

TRANSPLANTS FROM THE LATE GASTRULA STAGE

Embryos designated as late gastrulae are those having a very small yolk plug or a slit-shaped blastopore. These stages are very close to the end of gastrulation when the main organ anlagen of the embryo have been firmly determined (Spemann, 1918). Rectangular pieces of tissue were taken from the following regions and implanted into early gastrulae: (1) ventral presumptive epidermis, (2) presumptive medullary material, and (3) archenteric roof. Induction was observed only after implantation of presumptive medullary material or archenteric roof.

Plate 22, figure 8, shows a specimen which received an implant of archenteric roof; it illustrates at the same time a secondary medullary structure oriented in the transverse plane of the host. Incidentally, it should be stated that the secondary embryo may occur at almost any angle to the primary, although in the majority of occurrences the longitudinal axes of the two are approximately parallel. Similar relationships between primary and secondary embryos have been observed in *Triton* (Bautzmann, 1929). Sections of the above-mentioned embryo confirmed the existence of a well developed secondary medullary plate lying against the implanted material.

TRANSPLANTATION OF PARTS OF THE NEURULA

The following regions of the neurula were implanted into early gastrulae: (1) ventral epidermis, (2) medullary plate, and (3) chorda and somites. Induction was obtained with the medullary plate and chorda-somite implants only. In several series of experiments, morphologically well developed secondary medullary plates were not observed after implantation of medullary material. In other series of experiments from 25 to 40 per cent of the specimens developed externally visible secondary medullary plates.

Induction by medullary material has been observed in *Triton* (Mangold and Spemann, 1927; Mangold, 1928 and 1929).

DEVELOPMENT OF EXPLANTED ANLAGEN

THE PRECEDING EXPERIMENTS have shown that the dorsal and lateral blastoporal lips of the early gastrula, which during gastrulation become the archenteron roof, can so alter the development of presumptive epidermis that it becomes a secondary medullary plate. The fact that in normal development the process of gastrulation brings the organizer into a position beneath a portion of the ectoderm suggests that the normal medullary plate, like plates produced experimentally, may arise in connection with some influence transmitted from the archenteron roof. If it can be shown, however, that the presumptive medullary material develops into medullary plate when isolated from the rest of the early gastrula, it would seem that a substratum of archenteric roof is at least not the sole factor involved.

Bits of tissue were cut from selected positions on the embryo and maintained in Holtfreter's solution upon a substratum of beeswax. For each series of explants, several controls were kept in the same dishes. In order to allow sufficient time for development, explants were fixed after the controls had finished neurulation.

The following types of explants were prepared:

- A. Simple explants from the early gastrula
  - 1. Presumptive medullary material
  - 2. Presumptive epidermal material
- B. Simple explants from the late gastrula
  - 1. Presumptive medullary material
  - 2. Presumptive epidermal material
- C. Compound explants from the early gastrula
  - 1. Presumptive medullary with dorsal lip material
- D. Compound explants from the late gastrula
  - 1. Presumptive medullary material with archenteric roof

SIMPLE EXPLANTS FROM THE EARLY GASTRULA

Presumptive Medullary Material

Plate 23, figure 10 shows a typical example of presumptive medullary material from an early gastrula cultured *in vitro*. Certain of the cells have become elongate and closely packed, and the nuclei somewhat elliptical. No medullary folds are present; it is only in the morphology of the individual cells that there is a resemblance to medullary material. That a change in cell morphology has actually occurred is evident from a comparison with sections from the presumptive medullary region of the early gastrula (pl. 23, fig. 9). Low columnar cells apparently occur in the latter, but the absence of elliptical nuclei arranged with their axes parallel to one another, the looser aggregation of the cells, and their relatively cuboid form, indicate a less differentiated condition..

Erdmann (1931) found that in *Amblystoma* pure presumptive medullary material could undergo medullary differentiation *in vitro*. Holtfreter (1933*b*) could observe no such differentiation, except in an occasional cell; the latter phenomenon, he suggests, requires further investigation (p. 412).

Presumptive Epidermal Material

Presumptive epidermal explants are divisible into two groups; in some specimens a more or less definite amount of the tissue is composed of cells resembling medullary cells (pl. 23, fig. 12), whereas in others no trace of such differentiation is discernible (pl. 23, fig. 11).

"Medullary" differentiation in both the presumptive epidermal and the medullary explants was usually limited to a comparatively small region of the total explanted tissue (pl. 23, fig. 12). Sometimes a greater part of the material showed "medullary" differentiation (pl. 23, fig. 10). In all specimens at least a portion of the explants was composed of approximately cuboid cells bearing no resemblance whatsoever to medullary cells. These facts suggested that perhaps there was involved some unsuspected difficulty in estimating the extent of the presumptive epidermal material and that in this difficulty might lie the explanation of the occurrence of localized areas of "medullary" and non-medullary differentiation in the same explant. Experiments undertaken to test this possibility indicate that at least two disturbing factors must be considered in evaluating the results obtained.

In the first place, what degree of accuracy can be expected in estimating the boundary between presumptive epidermal and medullary materials? Nile blue sulfate was used to stain a single mark near the animal pole of ten early gastrulae. In seven of these the mark occurred on the transverse fold of the neurula or in the extreme anterior end of the medullary plate. In one animal the mark lay well within the medullary plate. In two the marks were found in the epidermis. Judgments concerning the boundary between presumptive medullary and epidermal materials are therefore fairly but by no means absolutely accurate. This may be attributed, at least to some degree, to variations in the eggs themselves.

A second possible source of difficulty should be considered in the preparation of explants from the presumptive medullary and epidermal regions. In vital staining experiments the chorion remains intact, but in explantation the chorion must necessarily be removed. The relatively soft egg then tends to flatten out and thus to alter topographical relationships ascertained by vital staining experiments.

Consideration of these technical difficulties suggests that the occurrence of "medullary" and non-medullary differentiation in the same explant may possibly be referred to the inclusion of portions of both presumptive epidermal and medullary materials in the explants.

SIMPLE EXPLANTS FROM THE LATE GASTRULA

Presumptive Medullary Material

Pure presumptive medullary material from the late gastrula, in contrast with similar material from the early gastrula, develops into a much thicker plate of densely packed and greatly elongated cells (pl. 23, fig. 13). The higher degree of differentiation is unmistakable. It appears that the association of this material with the rest of the embryo during gastrulation results in an increased capacity to differentiate medullary cells. The presence of the organizer alone is sufficient to produce this increased differentiative capacity as is shown by the experiments with compound explants.

Presumptive Epidermal Material

Presumptive epidermal explants from the late gastrula develop into hollow or partly solid masses of cells, without any signs of medullary differentiation.

COMPOUND EXPLANTS FROM THE EARLY GASTRULA

Presumptive Medullary with Dorsal Lip Material

Presumptive medullary material of the early gastrula, while it shows a tendency to differentiate into cells resembling medullary cells when maintained *in vitro* (pl. 23, fig. 10), stands in sharp contrast to similar material cultured in contact with dorsal lip tissue of the early gastrula (pl. 23, fig. 14). The more elongate cells in the latter form a thicker and more compact plate. The degree of differentiation approaches that already described for pure presumptive medullary explants from the late gastrula (pl. 23, fig. 13). Contact with the organizer alone thus produces histological changes in the medullary anlage similar to those which appear in normal gastrulation.

It is to be noted that no differentiation into chorda and somites is visible in the dorsal lip material in plate 23, figure 14. The ability to induce is thus apparently not dependent upon differentiative processes in the organizer. A similar independence between capacity to induce and histological differentiation has been observed in the presumptive medullary material of *Triton* (Mangold, 1929).

COMPOUND EXPLANTS FROM THE LATE GASTRULA

Presumptive Medullary Material with Archenteric Roof

Such explants develop the elongate cells already described for compound explants from the early gastrula and for pure presumptive medullary explants from the late gastrula.

DISCUSSION

THE ABILITY of presumptive medullary material of the early gastrula to differentiate elongate cells when cultured *in vitro* (pl. 23, fig. 10) suggests that a substratum of archenteric roof may not be the sole factor involved in medullary plate formation. Whether or not there is already present in the future neural material at this early stage of development a tendency toward medullary differentiation, depends entirely upon the significance of the elongate cells already described.

Cells of this shape are not limited exclusively to the medullary plate; they are, for example, to be found lining the blastoporal groove and in the early somites. At the present time we can only say that the presumptive medullary material after explantation into Holtfreter's solution is capable of differentiating elongate cells resembling medullary cells even before it becomes lined by the chordamesodermal substrate. In no circumstances could the explanted presumptive neural material, isolated from the chordamesoderm, develop a structure possessing the formal characteristics of the medullary plate. This, however, is hardly to be expected since the explants constitute only a fraction of the entire anlage. The presumptive medullary material has inherent capacities for medullary differentiation in the sense that at least some of its cells, independently of the organizer, can undergo certain histological changes involved in the production of the medullary plate.

The effects obtained by adding dorsal lip tissue (pl. 23, fig. 14) clearly indicate that whatever differentiative capacities may be present in the prospective nervous material are supplemented by factors connected with the chordamesoderm.

In contrast to its meager differentiative capacities indicated at the beginning of gastrulation, the medullary material of the *late* gastrula is capable of marked differentiation (pl. 23, fig. 13) even when isolated from chordamesodermal tissue. This may be explained as a result of close association with the chordamesodermal substratum during the period of gastrulation. When such association is produced experimentally by fusion of presumptive medullary plate of the early gastrula with dorsal lip, the presumptive medullary materials of the early and late gastrulae show more similar differentiative capacities (cf. figs. 13 and 14).

These data suggest that the development of the normal medullary plate may involve factors already present in the presumptive medullary material at the beginning of gastrulation as well as others connected with the organizer. The results of certain recent experiments are perhaps only apparently contradictory to this view. Thus Eakin (1933) could ascertain no medullary differentiation in the presumptive medullary material of *Triturus torosus* when normal gastrulation was prevented by filling the blastocoele with a gelatin mass. Holtfreter (1933*b*) obtained exogastrulae in the axolotl after removing the chorion in the blastrula stage. Embryos were obtained in which the ectoderm was almost completely separated from the entomesoderm, forming irregularly folded masses without any sign of medullary differentiation. Holtfreter concludes that the presumptive neural material of the early gastrula, without a mesodermal substratum, is incapable of any medullary differentiation whatsoever.

It should be recalled, however, that the experimental procedure and conditions of the work herein reported are different from those involved in the work of Eakin and of Holtfreter. Considering the fact that the differentiation of any tissue involves extrinsic factors to a greater or lesser extent, the results of the above workers are not, therefore, necessarily opposed to the suggestions presented in the present paper. A fuller treatment of the problem will follow in a later paper.

SUMMARY

1. Several recent theories concerning the factors involved in the formation of the medullary plate in urodeles are briefly characterized.

2. The method used in the present experiments involved the excision and cultivation in the blastocoele or *in vitro* of bits of tissue from embryos of *Triturus torosus,* at various stages of development.

3. The locations of the presumptive epidermal and medullary materials of the early gastrula were ascertained by the use of vital stain.

4. Of the parts of the embryo tested, well developed inductive capacities were found in the following:

   A. The dorsal and lateral lips of the early gastrula stage
   B. The dorsal and lateral blastoporal lips and the archenteric roof of the middle gastrula stage
   C. Archenteric roof and presumptive medullary material of the late gastrula stage
   D. Chorda-somite and medullary materials of the neurula stage

5. Normal configuration of the secondary neural tube is related to the normal arrangement of the chorda and somites.

6. Presumptive medullary material of the early gastrula, isolated before it comes into contact with the substratum of archenteric roof, is capable, under the conditions involved in the present experiment, of producing occasional elongate cells resembling the medullary type.

7. A. Elongate cells were observed in explants supposedly composed of "pure" presumptive epidermal material of the early gastrula.

B. From a consideration of the localized nature of such medullary differentiations, and the difficulties involved in estimating the boundary between presumptive epidermal and medullary regions, it is suggested that medullary differentiation in supposedly presumptive epidermal material may possibly be referred to encroachment upon the presumptive medullary region.

8. Presumptive medullary material from the early gastrula cultured in contact with dorsal lip of the blastopore exhibits a degree of differentiation more closely resembling that which occurs in explanted medullary material from the late gastrula.

9. The presumptive medullary material of the early gastrula appears to be capable of a certain degree of self-differentiation in the sense that, after explantation, some of its cells, independent of a chordamesodermal substrate, can undergo certain of the histological changes involved in the development of the medullary plate.

10. It is pointed out that conclusions of certain recent studies, apparently contradictory to the conclusions expressed in the present paper, are probably to be referred to different experimental procedures and conditions.

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EXPLANATION OF PLATES

All figures drawn with the aid of a camera lucida.

PLATE 22

Fig. 1. Locations of stains *(4–1; 18–15; 12–14)* in the early gastrula of *Triturus torosus*, no. 39, view of the left side. × *ca.* 17. *bp.*, blastopore.

Fig. 2. Locations of the stains *(4–1; 18–15; 12–14)* in the neurula of *Triturus torosus*, no. 39, view of the left side. × *ca.* 17. *bp.*, blastopore; *mp.*, medullary plate.

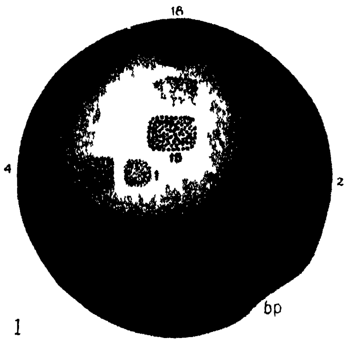
Fig. 3. Early stage in development of secondary embryo after implantation of dorsal blastoporal lip of the early gastrula in *Triturus torosus*, no. 833, view of the right side. × *ca.* 17. *nf.*, and *nf*^{2}., primary and secondary neural folds, respectively.

Fig. 4. Later stage in development of secondary embryo in *Triturus torosus*, no. 833, view of the right side. × *ca.* 17. *nt.*, and *nt*^{2}., neural tubes of normal and induced embryos, respectively.

Fig. 5. Cross-section through *Triturus torosus*, no. 833. × 25. *ar.*, archenteron; *ch*^{2}., implanted chorda; *nt.*, primary neural tube; *nt*^{2}., induced neural tube., *so*^{2}., implanted somite.

Figs. 6 and 7. Cross-sections through *Triturus torosus*, no. 833. × 45. *ch*^{2}., implanted chorda., *nt*^{2}., induced neural tube; *so*^{2}., implanted somites.

Fig. 8. Secondary medullary plate after implantation of archenteric roof of the late gastrula in *Triturus torosus*, no. 252, ventral view. × *ca.* 17. *mp*^{2}., induced medullary plate; *nf.*, primary neural fold.



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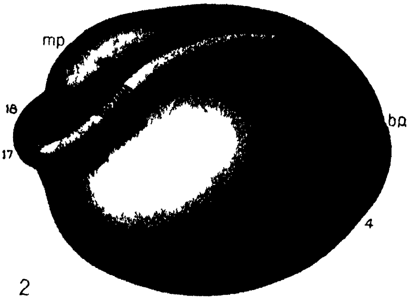

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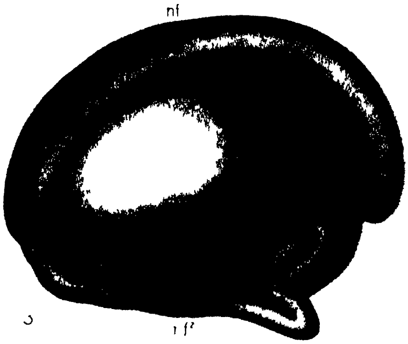

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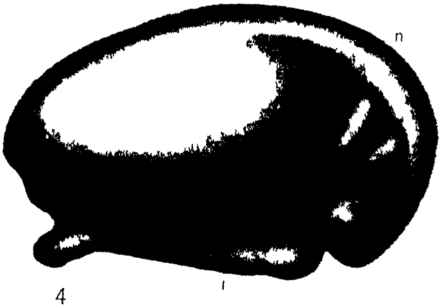

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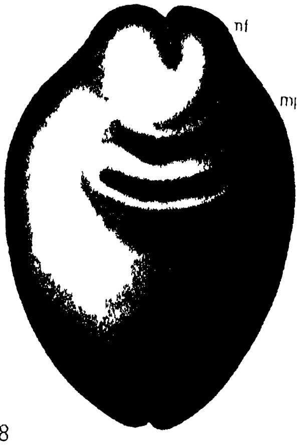

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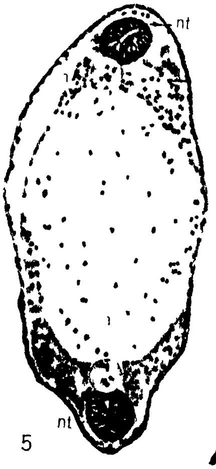

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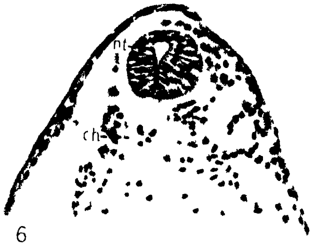

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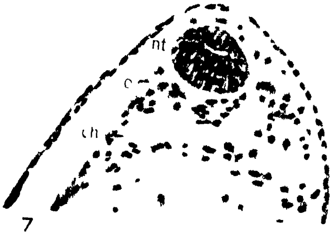

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PLATE 23

All figures except figure 9 are from objects maintained *in vitro* until control embryos had completed neurulation.

Fig. 9. Longitudinal section through presumptive medullary material of the early gastrula of *Triturus torosus*. × 266. *ch.*, chorion.

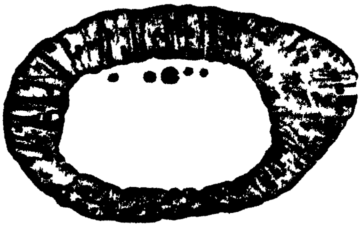
Fig. 10. Section through an explant taken from presumptive medullary material of the early gastrula of *Triturus torosus*, no. 576–2. × 76.

Fig. 11. Section through an explant taken from presumptive epidermal material of the early gastrula of *Triturus torosus*, no. 814–4. × 76.

Fig. 12. Section through an explant taken from presumptive epidermal material of the early gastrula of *Triturus torosus*, no. 675-1. × 76.

Fig. 13. Section through an explant taken from presumptive medullary material of the late gastrula of *Triturus torosus*, no. 642–4*c*. × 76.

Fig. 14. Section through a compound explant composed of presumptive medullary and dorsal blastoporal lip materials of *Triturus torosus*, no. 578–1*y*. × 76. *dl.*, dorsal blastoporal lip material; *mp.*, medullary material.



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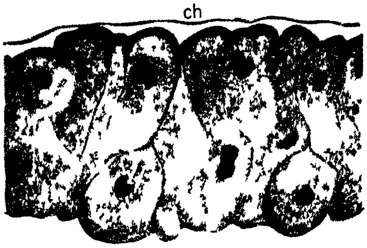

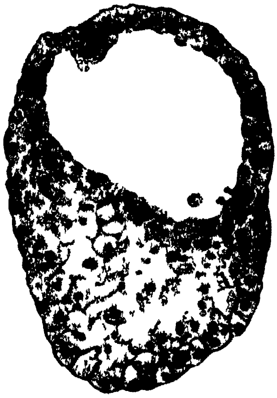

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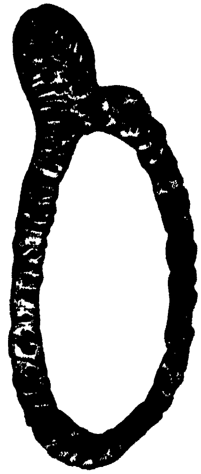

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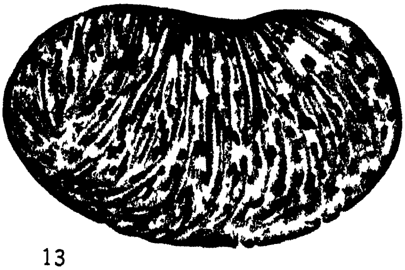

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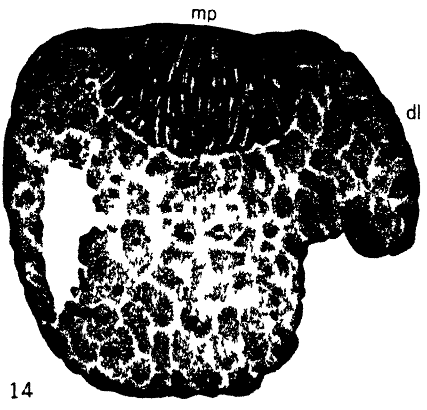

14

THE EFFECTS OF METHYLENE BLUE AND OTHER OXIDATION-REDUCTION INDICATORS ON EXPERIMENTAL TUMORS

BY

MATILDA MOLDENHAUER BROOKS

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THE EFFECTS OF METHYLENE BLUE AND OTHER OXIDATION-REDUCTION INDICATORS ON EXPERIMENTAL TUMORS

BY

MATILDA MOLDENHAUER BROOKS

INTRODUCTION

THESE EXPERIMENTS* were done to see whether the oxidation-reduction indicators could be used to cause regression of experimental tumors in animals.

They were begun in the spring of 1930 under a grant from the Medical Research Fund for Cancer of the University of California Medical School, and continued until the spring of 1933 under additional grants from the Christine Breon Fund and the Cancer Fund established by a friend, of the same school, the Board of Research of the University of California, and the National Research Council of Washington, D. C. These acknowledgments are gratefully made. The experiments were done with the assistance of Miss Elizabeth Wood and Miss Virginia Condit.

The theory on which these experiments were based has been given in the studies by W. M. Clark (1922) on oxidation-reductions and by Warburg (1923) on the metabolism of normal and tumor tissues. Warburg established the fact that tumor tissue is characterized by abnormal carbohydrate metabolism, obtaining most of its energy from glycolysis rather than from respiration. If, therefore, one were able to produce respiration in tumor tissue at the expense of glycolysis, it might be possible to change the tissue into normal growth. In order to see if this could be brought about, methylene blue was used, because it increases the rate of O_2 consumption and therefore the rate of metabolism. This property of methylene blue was first noted by Palladine (1911), then by Heymans and Maigre (1922), Heymans and Heymans (1922), and others. Genevois (1928) first ascribed this increased O_2 consumption to the catalytic action of the dye in experiments with *Chlorella*. Shortly afterward, Harrop and Barron (1928) came to the same conclusion.

In order to see whether the redox potential of methylene blue caused the effect of this dye, Genevois (1928) inquired into the relation between the potential and the rate of O_2 consumption, showing that those dyes which have a low redox potential (Rapkine, Struyk, and Wurmser, 1929) are not so effective as those whose potential is in close proximity to that of methylene blue. The studies of Barron and Hoffman (1930) on marine eggs also show this and their conclusions are in agreement with those of Genevois in ascribing the catalytic action of the dye to its position on the redox scale.

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* Preliminary report in the Proc. Soc. Exp. Biol. and Med., 30:1001, 1933. In a review of this paper (Am. Jour. Cancer, Sept., 1933) W. H. Woglom comments that injections of Ringer's solution would have produced the same results as those of methylene blue. In the preliminary report it was not explicitly stated that controls included sets both with and without injections of Ringer's. In this full report it will be seen that Woglom's assumptions were incorrect and his explanation of my results therefore not valid. Even without specific mention of the controls used, the fact that the other four dyes gave no promising results is sufficient to negate Woglom's criticism.

Therefore it seemed appropriate to use not only methylene blue but also some of the other indicators for comparison, to see whether the tissue would respond to the "poising" effect (Clark, 1922) of the various indicators at different levels, or whether that of methylene blue alone would be effective, if at all.

EXPERIMENTS

THE DYES SELECTED covered the range of easily reducible indicators whose E_0' is strongly positive, to easily oxidizable indicators having a negative E_0'. Those used in these experiments, together with their E_0' values and their designations by number, were as follows:

No.	Indicator	E_0'	At pH	Brand
1	0-chloro phenol indophenol	+.1966	7.45	LaMott Chemical Co.
2	0-cresol indophenol	+.1609	7.52	LaMott Chemical Co.
3	methylene blue	−.003	7.49	Merck's medicinal
4	neutral red	−.370	7.5	Coleman & Bell
5	brilliant cresyl blue	+.020 +.033	7.5 7.42	Grübler's

The *calculations* for the first three indicators were taken from Clark's (1922) results; no. 4 and no. 5 from Rapkine, Struyk, and Wurmser (1929); no. 5, second figure, from Cohen and Preisler (1931).

This report comprises two sections. In Section 1 all five oxidation-reduction indicators were used, while in Section 2 methylene blue only, which was found the most effective, was used. The two sections will be discussed separately.

BREEDS OF ANIMALS

Rats came from the New York State Institute for Malignant Diseases. Mice came from the Hooper Foundation of this University; the Memorial Hospital of New York City—a strain carrying a spontaneous mammary carcinoma, sent by Dr. Bagg; Dr. Millard Marsh, the New York State Institute for Malignant Diseases—strain 3; the Roscoe B. Johnson Memorial Laboratory, Bar Harbor, Me.—an inbred brown strain.

Since we had difficulty in obtaining litters with the last two breeds, the results reported will deal with the "Hooper" and "Memorial" mice only.

KIND OF TUMOR

Section 1.

Rat tumor no. 1 from the N. Y. S. I. M. D.

Mouse tumor no. 1A, *spontaneous* tumor from "Memorial" mice.

Section 2.

Rat tumor no. 2, Crocker Institute, no. 256/94A.

Mouse tumor no. 2A, Crocker Institute, Mouse sarcoma 180/328B.

My thanks are extended to all the institutions above mentioned for their courteous coöperation; to Dr. Sundstroem, of the Department of Biochemistry, University of California, for supplying the first rats, and for other courtesies; and to Dr. K. F. Meyer, of the Hooper Foundation, for encouragement and support.

METHODS OF IMPLANTATION AND TREATMENT

Implantations were made into three-month-old animals. Small pieces of minced tumor tissue taken from the actively growing area of a tumor were implanted subcutaneously by means of a trochar under aseptic technique.

TABLE 1

IMPLANTATIONS AND RESULTS

Animals	Total number inoculated	Takes (number)	Nontakes (number)	Died from other causes	Takes (per cent)	Nontakes (per cent)
Tumor no. 1—rats	620	390	210	20	63	28
Tumor no. 1A—mice	267	90	148	29	34	51
Tumor no. 2—rats	300	282	14	4	94	5
Tumor no. 2A—mice	160	114	30	16	71	19

When the tumor had grown to about 1 cm. in diameter in rats, or 0.5 cm. in mice, injections of one of the dyes were begun. The time necessary to produce tumor growth large enough for treatment to be begun varied with the different tumors and the size of the transplants, which could not be accurately gauged, even though attempts were made to keep the amount implanted close to 1 cu. mm. In the rats, tumor no. 1 required about three weeks; in the mice, tumor no. 1A, about one month, to attain the size appropriate for experiment. The Crocker tumor of rats and mice, no. 2 and no. 2A, grew faster, and required about two weeks.

The indicators listed above were used in the form of freshly made aqueous solutions whose concentration was 0.01 per cent for rats and 0.001 per cent for mice. Ringer-Tyrode's solution without glucose (hereinafter called "Ringer solution") was used as a solvent, the dry dye being first dissolved in a few drops of distilled water, and the Ringer solution then added. These solutions were sterilized at 100° C for one hour. The precipitate thus formed was redissolved by bubbling in CO_2 from pressure tanks. Since Ringer solution is well buffered at a pH of 7.5, it was assumed that neither the dye nor the CO_2 introduced any significant change in pH. This method of making and using the solutions obviates error caused by precipitation or deterioration of the dyes. Control injections were made with 0.85 per cent NaCl and with Ringer solution.

The amount of dye injected was governed by the size of the tumor, which in the later experiments was measured by calipers, twice or thrice weekly. This is admittedly a crude measure, since the tumors were often far from spherical; in such experiments the longest diameter was recorded. In general

0.2 cc. of solution was injected for each 0.5 cm. of tumor diameter. The amount could not be made proportional to the volume of the tumor, for example, 0.2 cc/ (0.5 cm.)^3, because the larger amounts of dye would have been toxic, especially to the mice. In small doses all the dyes used have been found nontoxic for tissues of all kinds. The largest dose in rats was never more than 1 cc.; in mice, 0.5 cc. All injections were limited as far as possible to rapidly growing, nonnecrotic regions of the tumor. They were given at first three times a week and later six times a week. The more easily reducible dyes were found to have been almost entirely reduced soon after injection; theoretically, however, some dye would always be present in the oxidized state, and a continuous flux or cycle of oxidation and reduction would be going on.

TABLE 2

SECTION 1. RATS

Treatment	Total number	Died	Regressed	Percentage of regressions	Days to regress	Days to death
Untreated	123	52	71	58	21	18
Ringer's	77	36	42	55	20	16
Dye no. 1	7	3	4	57	41	36
Dye no. 2	83	36	46	55	20	21
Dye no. 3	12	4	8	67	13	21
Dye no. 4	58	28	30	59	17	14
Dye no. 5	24	10	14	58	22	17

It is to be noted that much of the dye remained localized in the tumor, as shown by the color of the tumors examined at autopsy. When the dye was in the reduced form, the color reappeared on exposure of the cut surfaces to air.

RESULTS

The two sections into which these experiments are divided differ in the number of dyes used and in the types of tumor studied. More satisfactory tumors for both rats and mice were employed in Section 2.

Mouse tumor no. 1A (Section 1) was satisfactory in that it *never produced any regressions,* but it required a long time to grow. Mouse tumor no. 2A (Section 2) gave some regressions, but the tumor grew very fast. Spontaneous regressions (differing from those in which the tumor grew to an appreciable size before disappearing) were found in all groups, both treated and untreated, to the extent of about 10 per cent. In these the tumor always remained very small, never exceeding about 0.5 cm. in diameter, and then disappeared. Such cases are not included in the counts made of either treated or untreated animals as reported in any of the tables accompanying this paper.

SECTION 1

Rats.—In these experiments (table 2), which are exploratory, all the dyes enumerated were used. The controls consisted of tumors to which no treatment was given and those injected with Ringer alone.

Since the percentage of regressions was so high with this strain of tumor, the results are not very conclusive. All the dyes except no. 3 allowed a proportion of regressions which did not differ significantly from that occurring among the untreated animals or among those receiving Ringer solution alone. When dye no. 3 was used there were 66 per cent regressions as compared with 54 to 59 per cent in all other categories. This difference is significant because of corroboration in Section 2. Column 5 shows the number of days required for regression, which was less with no. 3 than with the other classes. Column 6 shows the number of days until death of the animals dying as a result of the tumor. While animals treated with dye no. 3 show longer survival than the untreated, this difference does not seem statistically significant; whereas dye no. 1 was less toxic than no. 3 in the number of days that the animals lived, this advantage is counterbalanced by the longer time it required for regressions to be completed (column 5). The other dyes did not produce any change in the percentage of regressions, which differed greatly from those in the untreated animals.

TABLE 3
SECTION 1. MICE

Treatment	Total number	Regression	Days to death
Untreated	23	0	21
Ringer's	11	0	20
NaCl	12	0	11
Dye no. 1	2	0	38
Dye no. 2	22	0	13
Dye no. 3	9	3	38
Dye no. 4	2	0	9

TABLE 4
SECTION 2. RATS

Treatment	Total number	Died	Regressed	Percentage of regressions	Average maximum size at death	Average maximum size at regression	Days to death
Untreated	57	53	4	7	6.6	1.7	49.4
Ringer's	31	28	3	9.6	5.6	2.1	46
Dye no. 3	96	54	42	44	4.7	1.5	58.8

Mice.—There was a high percentage of "nontakes" among the mice, but the "takes" never regressed, as did those of the rats. The time required for the development of this tumor varied, being usually one to two months, but sometimes three to four months, as contrasted with the rat tumor, which developed in a few weeks.

Of the ninety mice which developed tumors (table 3) none regressed except those treated with dye no. 3. Although the number used is small, further corroboration makes this significant. Column 3 also shows the favorable results of no. 3 in the length of survival of animals having tumors and receiving treatment.

The small percentage of takes with this tumor made it difficult to obtain animals in number sufficient for statistically conclusive tests. The long period required for development of the tumor also reduced the experimental yield.

TABLE 5

SECTION 2. MICE

Treatment	Breed	Total number	Number of regressions with tumor	Days to death	Average maximum size at death
Untreated	Hooper	21	2 with no. 2	26	2.4
	Memorial	10	0 with no. 2	24	2.4
	Memorial	23	0 with no. 1	22	2.7
	Total regressions = 3.7 per cent				
Ringer's	Hooper	2	0 with no. 1	...	......
	Memorial	9	0 with no. 1	20	......
	Memorial	17	1 with no. 2	37	1.7
	Hooper	2	0 with no. 2	38	1.9
	Total regressions = 3 per cent				
Dye no. 3	Hooper	21	4 with no. 2	30	1.4
	Memorial	9	3 with no. 1	38	1.7
	Memorial	37	10 with no. 2	32	1.6
	Total regressions = 25 per cent				

However, the results with dye no. 3 with mice, combined with similar results with rats, seemed sufficiently significant and promising to justify continuance of experiments with this dye on a more extensive scale, as described in Section 2.

SECTION 2

In this section Crocker tumors which gave a high percentage of takes and a low percentage of regressions were used, so that significant results could be obtained in a comparatively short time. The tables accompanying this section include some data from experiments which were not completed at the time the preliminary paper was published, and there are therefore a few changes in the general summaries.

Rats.—Table 4 shows the results with rats inoculated with the Crocker tumor. Column 5 gives the average maximum size of the tumor in the animals that died as a result of the tumor. Column 6 is the average maximum size of the tumor of the animals with tumors that regressed. Column 7 is the average of the number of days until death of the animals dying as a result of the tumor. These results show that dye no. 3 was effective in keeping the size of the tumor smaller and in prolonging the life of the animal.

Column 4 shows the percentage of regressions of all three sets. The untreated and Ringer's averaged 8 per cent regressions, while the dye-treated produced 44 per cent. This shows definitely that dye no. 3 (methylene blue) acts favorably in causing regression. The tumor becomes reduced in size, sloughs off, and heals up.

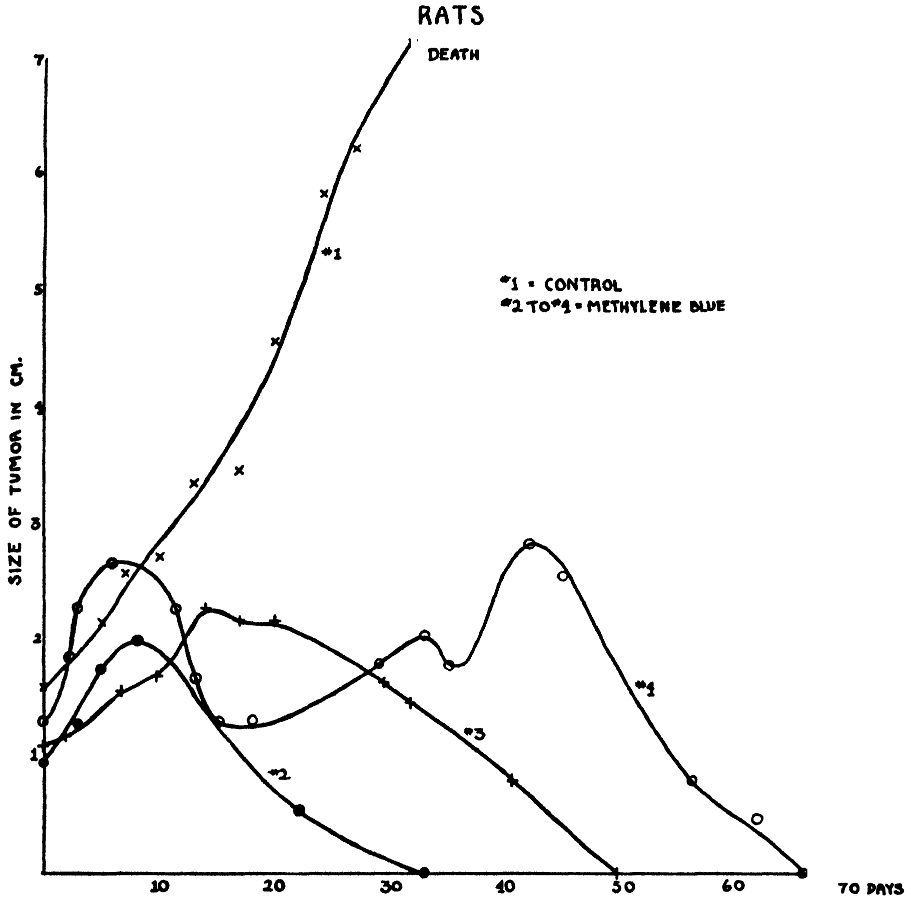

Fig. 1. Change in size of tumor (ordinates) of rats during days (abscissae). Curve 1 is untreated control; curves 2–4 represent tumors treated with methylene blue.

Mice.—Table 5 shows the results with both tumor no. 1 and tumor no. 2 when treated with dye no. 3. Untreated animals and those receiving Ringer's solution showed regressions in about 3 per cent of 84 cases, while those treated with dye no. 3 produced 25 per cent out of a total of 67 cases. The variation in the percentage of regressions with different strains of mice and different tumors may or may not be of any significance, but even the least successful of the three groups of treated animals shows a proportion of regressions which is five times as high as that of the controls.

The length of life of the animals having the two tumors varied. Tumor no. 1 killed the animals faster than tumor no. 2. Dye no. 3 lengthened the life of the treated animals over that of the untreated. The Hooper mice seemed to be more resistant than the Memorial. Tumor no. 1 never gave regressions in the controls. The average size of the tumor in the untreated animals was larger at death (its maximum) than with either the Ringer's solution or dye no. 3.

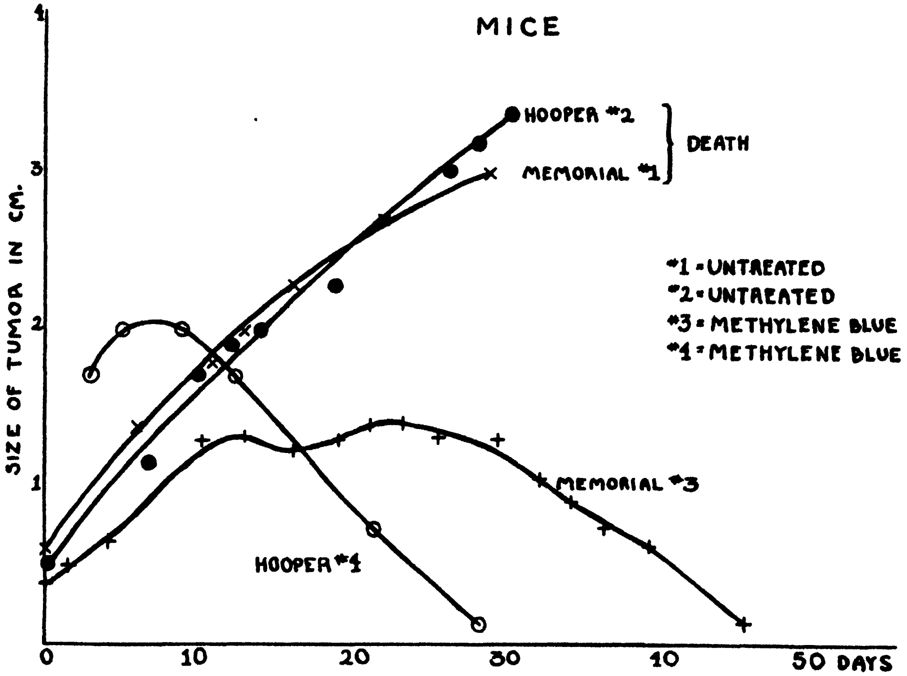

Fig. 2. Change in size of tumor (ordinates) of mice during days (abscissae). Curves 1 and 2 are untreated controls; curves 3 and 4, tumors treated with methylene blue.

In both rats and mice, regressions were usually preceded by crater formation, sloughing, and healing up with scab formation, which eventually disappeared under growth of hair.

It might be thought that the effect of methylene blue in causing regression of tumors could be ascribed to the toxicity of the dye. But this seems very improbable, since it has been shown by several workers that the concentration used in this experiment is in general not toxic. Barron and Hoffman (1930), using three times the concentration used by the writer, found no deleterious effect on the O_2 consumption of starfish eggs during the course of their experiments. The writer (1933) has used the same concentration of methylene blue in experiments *in vitro* with no apparent injury to fertilized sea urchin and starfish eggs, which passed successfully through all the stages of development of the embryo to the pluteus stage.

Figures 1 and 2 show the growth of representative individual tumors in control and treated rats and mice respectively. In figure 1, curve 1 is typical of untreated tumors, curves 2 to 4 of treated. In figure 2, curves 1 and 2 represent untreated Hooper and Memorial mice tumors, while curves 3 and 4 represented treated tumors.

These results are in agreement with those obtained by Roffo (1931), published after this investigation was under way. His methods were different inasmuch as he subjected the tumor tissue to the dyes before transplanting, then noted the percentage of takes.

CONCLUSIONS

SINCE THE PERCENTAGE of regressions obtained with methylene blue treatment is significantly greater than that of the controls (with or without injections of Ringer solution, or under treatment with other dyes), these experiments show that methylene blue inhibits the growth of transplantable tumors. The interpretation is made that this dye "poises" the tissue at a redox potential compatible with normal growth. Through the catalytic action of the dye, O_2 consumption is increased, glycolysis discouraged, and normal growth produced.

SUMMARY

OUT OF five oxidation-reduction dyes which were used as injections into transplantable tumors in rats and mice, it has been shown that methylene blue is the only one which is efficient in causing regressions. Methylene blue gave 44 per cent regressions in rats as compared with 8 per cent in the controls (including both the untreated and those receiving Ringer solution); and 25 per cent in mice, as compared with 3 per cent in the controls.

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UNIPOLAR INGRESSION IN TRITURUS TOROSUS: A HITHERTO UNDESCRIBED MOVEMENT IN THE PREGASTRULAR STAGES OF A URODELE

BY

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IN THE COURSE of vital-staining experiments in 1932, it was noted that certain marks placed on the ventral side of eggs in early cleavage stages had disappeared by the time that gastrulation began. This observation was not followed up at that time; it was tentatively explained as a result of the rapid fading of vitally stained marks on the yolk-rich lower part of the egg. In 1934 the disappearance of marks placed on the ventral part of the zygote was confirmed. Marks made on the vegetative field of the early *gastrula* remain visible for six or more days; it seemed remarkable that similar marks placed on the vegetative field of the *zygote* should sometimes vanish within thirty hours.

Further investigation later revealed the results herein described. Briefly stated, it was found that the vegetative material of the zygote undergoes a movement into the interior of the egg throughout the cleavage or blastula stage. The yolk-laden ventral part of the egg, hitherto regarded as more or less inert and motionless (vegetative), is subject to an active displacement, in which the material exposed on the ventral surface of the *zygote* penetrates into the central portion of the *blastula.*

Such unipolar ingression has been recorded in a number of invertebrates. Treadwell (1901) and Shearer (1911) have described it in annelid worms. It is found in the Nematoda and Mollusca and is present in the Arthropoda and the Coelenterata, occurring in *Tima* and *Obelia* among the hydroids; it is found also in certain Anthomedusae, Scyphomedusae, and Leptomedusae (Richards, 1931).

A survey of the literature, including many of the classical works of embryology as well as recent papers, has thus far failed to disclose a description of unipolar ingression in any vertebrate. Brachet (1903), in considering the ways by which the blastocoele changes its form during blastulation, suggested "invagination of the vitelline cells" as a possibility (p. 15), but dismisses this type of movement as improbable: "On ne comprend pas comment cette masse compacte de cellules vitellines pourrait s'invaginer sans que la surface inférieure de l'œuf devienne plane ou déprimée..." (pp. 15–16). The data given in the present paper demonstrates that an inward movement of vegetative material does occur during blastulation and that it plays an important part in changing the shape of the blastocoele by producing the convexity which appears in its floor (see fig. 5, pl. 24).

Among the more recent papers dealing with the pregastrular stages of Amphibia are those of Smith (1922, 1926) on *Cryptobranchus alleghenienşis,* and Votquenne (1934) on *Rana fusca.* In neither of these is there any mention of pregastrular unipolar ingression. Votquenne (1934) applied vital stains to the egg of *Rana fusca* in early cleavage stages. Although the results he obtained after staining the upper portions of the egg are very similar to those I have obtained this year with similar stages of *Triturus torosus,* there is no reference to unipolar ingression.

MATERIALS AND METHODS

The method of localized vital-staining used was that described by Vogt (1925, 1929). The zygote of *Triturus torosus* was removed carefully from the egg jelly, leaving the chorion intact, and was stained with nile-blue sulfate and neutral red. Eggs were maintained in Holtfreter's solution (1931), upon a substrate of beeswax. The positions of the marks were recorded with the aid of a camera lucida. The rotary disc described by Daniel and Burch (1933) was used in drawing marks placed on the side of the egg. A new device, which forms a useful supplement to the rotary disc, was constructed in order to obtain *ventral* views of the egg without the necessity of inversion. In this device certain of the parts used in the Daniel-Burch Rotary Disc were assembled as shown in figure A.

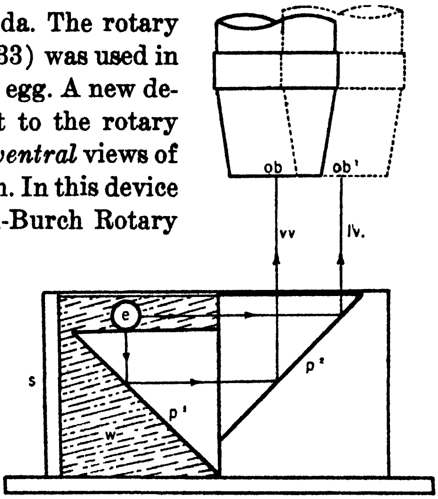

Fig. A. Diagram of apparatus used in order to obtain a ventral view of the egg without the necessity of inversion.

b., base; *e.*, egg; *lv.*, course of rays which give lateral view of the egg; *ob.* and $ob.^1$, objective of microscope in position for ventral and lateral views, respectively; $p.^1$ and $p.^2$, prisms; *s.*, side of reservoir; *vv.*, course of rays which give a ventral view of the egg; *w.*, water filling cavity of reservoir so that the egg is completely immersed.

APPARATUS

The apparatus consists essentially of two small 90° prisms ($p.^1$ and $p.^2$) juxtaposed as shown in figure A. The sides (*s.*) of the device were composed of sections cut from microscope slides; these form a reservoir which is filled with water (*w*). The horizontal face of one prism ($p.^1$) supports the egg (*e.*), which is observed by focussing the objective of the microscope (*ob.*) on the horizontal face of the second prism ($p.^2$). It is to be noted that both a ventral view (*vv.*) and a lateral view (*lv.*) are obtainable by simply altering the position of the objective as shown in figure A. Since the egg (*e.*) is supported on the surface of one prism ($p.^1$), and since both prisms are in direct contact, refraction is reduced to a minimum. It is further to be noted that the present device is not capable of the rotary movement obtainable with the Daniel-Burch Rotary Disc. It is therefore only suitable for experiments in which rotation of the egg may be accomplished by instruments such as hair-loops.

DEMONSTRATION OF UNIPOLAR INGRESSION

Triturus torosus, No. 16T, 1934, represents a typical example of the results obtained. Four successive views are represented in plate 24, figures 1–4. On February 16, at 5:30 P.M., one large circular red mark and two smaller blue ones were placed on the *ventral* surface of the zygote (fig. 1).

Five and a half hours later (11 P.M.) the egg was in the four-cell stage (fig. 2). The cleavage furrows extended completely around the egg to the vegetative pole, distorting the original shapes of the marks. Marks 2 and 3 were lengthened, and parts of them passed out of view into the cleavage furrows. Mark 1, now lobate in outline, coincided with the intersection of the first two cleavages, and, like the blue marks, seemed to have been partly drawn into the furrows. It is to be noted that marks 1 and 2 have moved closer together; the unstained area which separated these marks in the zygote stage (fig. 1) was no longer present in the four-cell stage (fig. 2).

On February 17, at 11:40 A.M., the marks were radically altered in form (fig. 3). As compared with their original condition (fig. 1), they had been reduced in size. Mark 1 was somewhat stellate in form; marks 2 and 3 were spindle-shaped. All the marks passed out of view into the cleavage furrows.

Figure 4 shows the marks as seen about five hours later (4:30 P.M.). Mark 1 was very irregular in shape and followed several cleavage furrows. Marks 2 and 3 were still more attenuated than at the preceding observation (fig. 3).

At the following observation, two hours and ten minutes after the second, the marks were still further reduced in size and had again altered their shapes. Mark 1, now very slender, followed the course of several cleavage furrows. Marks 2 and 3 had lost their former monaxial form and were now triaxonic.

The last external observation was made on February 17, at 10:15 P.M., when the egg was in the mid-blastula stage; only two tiny and very faint traces of color were visible externally, one blue, the other red.

The egg was now bisected (fig. 5) by means of a needle-knife and a hair-loop. In the middle and the upper part of the thick floor of the blastula were a number of spheroidal yolk cells colored red or blue or both red and blue.

It may be suggested tentatively that the ingression is accomplished by means of the cleavage furrows which carry portions of the vegetative surface into the interior of the egg. Careful observation under higher magnifications of the binocular microscope reveal that the stained areas extend into any furrows which may cut through them. Furthermore, as the ventral surface of the egg undergoes cleavage the vitally-stained marks tend to lie adjacent to the cleavage furrows (figs. 3–4, pl. 24).

A fuller treatment of the mechanism by which pregastrular unipolar ingression is accomplished will be presented in a later paper.

SUMMARY

1. Localized vitally-stained marks placed on parts of the ventral (vegetative) hemisphere of the zygote *(Triturus torosus)* are altered in shape and diminished in extent during the course of cleavage and blastulation. Such vitally-stained marks are later found in the interior of the middle or late blastula stage, comprising the upper part of the floor of the blastula.

2. It follows from the foregoing data that a unipolar ingression occurs in pregastular stages of development in the egg of the urodele, *Triturus torosus.* To the best of the author's knowledge, this is the first description of such a process in a vertebrate.

3. It is suggested tentatively that pregastrular unipolar ingression is accomplished by the inward-moving cleavage furrows which carry parts of the vegetative surface into the interior of the egg.

4. An apparatus is described which makes possible both a ventral and a lateral view of the egg without the necessity of inversion, and with a minimum of refraction.

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EXPLANATION OF PLATE

PLATE 24

All figures were drawn with the aid of a camera lucida, and represent views of the same egg, *Triturus torosus, No. 16T, 1934.* For complete description see text.

Locations of vitally stained marks *(1–3)* are indicated by red for neutral red, and blue for nile-blue sulfate.

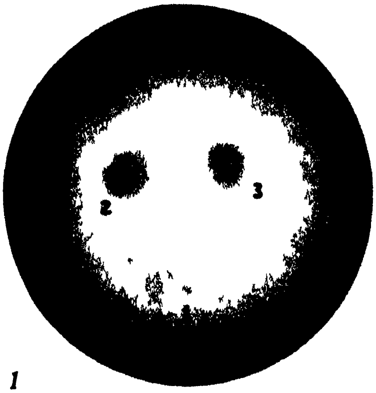
Fig. 1. Ventral view of the zygote stage shortly after staining. February 16, 5:30 P.M.

Fig. 2. Ventral view five and a half hours after figure 1 was recorded. February 16, 11 P.M.

Fig. 3. Ventral view about eighteen hours after figure 1 was recorded. February 17, 11:40 A.M.

Fig. 4. Ventral view about twenty-three hours after figure 1 was recorded. February 17, 4:30 P.M.

Fig. 5. View of one-half of the bisected blastula showing internal locations of stained materials as a result of ingression; recorded about twenty-eight and three-fourths hours after figure 1. February 17, 10:15 P.M.



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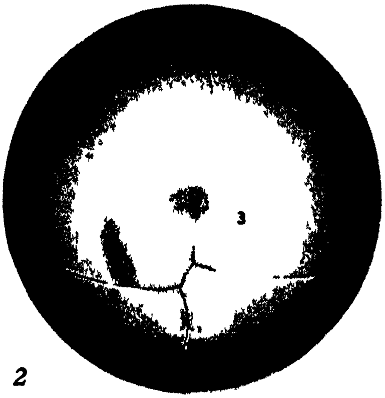

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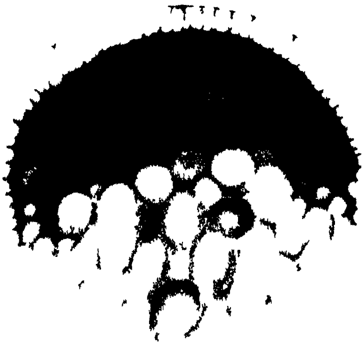

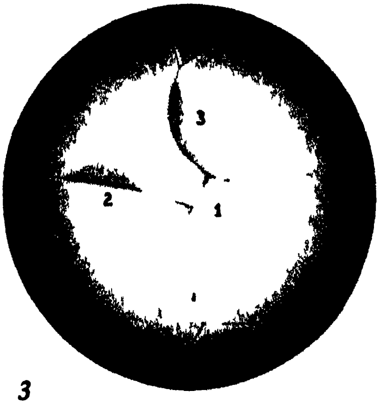

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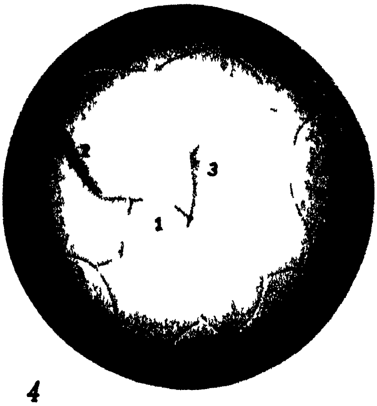

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THE CIRCULATION OF BLOOD IN AMMOCOETES

BY

J. FRANK DANIEL

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THE CIRCULATION OF BLOOD IN AMMOCOETES

BY

J. FRANK DANIEL

INTRODUCTION

THE DISCOVERY of the circulation of blood by Harvey is regarded as one of the outstanding deductions made by man (see Leake, 1928). Its importance at the time was derived from the fact that it gave a reasonable answer to a series of questions. But an understanding of the full import of circulation was not possible in Harvey's day. Indeed, at our own time we cannot boast of a complete understanding of all that is involved in circulation. Notwithstanding that fact, our concept includes more than the mechanics of circulation. We see the blood channels as a great transportation system, reaching the tissues and the individual cells through miles of capillaries (Krogh, 1929), and the blood stream moving through these channels as a common carrier providing the living organism with such essential supplies as oxygen and nutriment, hormones and vitamins. In addition to the contribution which the circulating blood makes to the upbuilding of the individual, it provides a way by which certain waste products, such as carbon dioxide and nitrogen, are transported to organs for elimination from the body. Furthermore, by means of certain cells which are free to pass out into the tissues and later reënter the blood stream, it removes debris of a solid sort. In the active defense of the body an element of great importance is the serum, contained within the fluid part of the blood, by which an active immunity may be built up. With all these important functions to perform, the circulatory system is to be looked upon as a great integrating system (Cannon, 1930), without which higher organisms would be impossible (Henderson, 1928).

My work concerns itself with the structure and development of the organs of circulation, and the movement of blood in the head and pharynx of the lowliest of vertebrated animals, Ammocoetes, the larva of the cyclostome, *Entosphenous tridentatus*. Anyone who studies this, an almost transparent animal, by the use of a modern microscope, will but wonder at the astute observations and deductions made by Harvey, and will willingly accord to him full credit for his demonstration of the circulation of blood.

METHOD

These interesting larvae occur in abundance in the mouths of streams along the western Pacific slopes, and may be taken from the sand near the shore after they have reached seven millimeters in length. The eggs and younger stages occur on or near the surface of the sand in deeper pits or nests farther out in the quiet waters of the stream. All stages may be kept under laboratory conditions, and when needed they may be anaesthetized in a one-tenth per cent solution of chloretone. These anaesthetized animals may be placed in a small amount of water, in any desired position, in a narrow groove made in paraffin on a glass slide. To prevent evaporation, a cover slip is placed over the groove and additional water may be added if or when the circulation falters.

THE HEART

The heart in a young larva of from three to five millimeters in length has only two parts, which are readily observable in the living animal. These are a small atrium with the form of an inverted U, by the wide mouth of which it joins the sinus venosus, and a balloon-shaped ventricle which at this stage is much larger than the atrium. In a larva from seven to eleven millimeters long, all parts of the heart, sinus venosus (*sv.*, fig. 1), the single atrium (*at.*), the single ventricle (*vn.*), and the conus arteriosus (*co.*) are readily observable, lying just in front of the large liver (*l.*).

The sinus venosus (*sv.*, figs. 1–3) in external form is like a funnel with the mouth abutting against the liver, and the apex hollowed out in the atrial wall, pointing forward to the left of the animal. If observed with the ventral side turned a little to the left, the thin walls of the sinus are evident and its internal form can be determined by the column of blood contained within it. The modifications which this column undergoes as the sinus fills and partly empties serve to indicate the changes of the internal form of the sinus itself. When the sinuatrial aperture is closed (*sv.*, fig. 2) and the column of blood is arrested momentarily, its form is that of a truncate cone. When the aperture opens, the column is conical in form.

The extent of this collecting sac is best made out by the great vessels which enter it. A large hepatic vein from the liver empties directly into it, and the entrance of the inferior jugular (*ij.*, figs. 1–4, 8) at the tip of the liver marks its anterolateral (right) angle. The left superior jugular (anterior cardinal, *ac.*, fig. 3) serves to indicate the posterolateral (left) angle. The sinus thus delimited by the mouths of these great vessels extends obliquely forward to the left to join the atrium at the sinuatrial junction.

The valves (*sa.*, fig. 2) guarding the aperture between sinus and atrium consist of a lateral and a median fold. The lateral fold is attached to the posterolateral wall of the atrium, and when the aperture is open, extends this wall well into the atrial cavity.

The atrium, when filled with blood (*at.*, fig. 3), is a relatively large sac located on the left side of the midline, and in a specimen ten millimeters or more in length, reaches almost as far forward as the anterior tip of the ventricle. Posteriorly it extends well beyond the ventricle. When empty and in atrial systole (fig. 2), its form is changed so that the anterior tip is bent backward and inward and the posterior part extends forward and toward the median line.

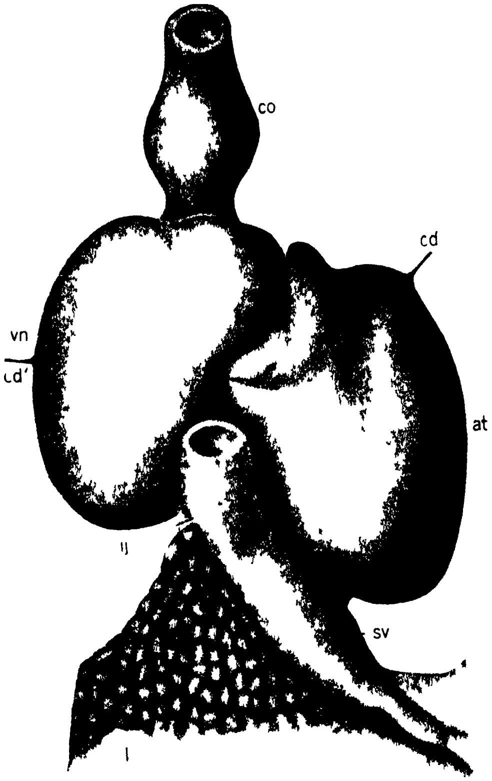

Fig. 1. Heart, 10 mm., Ammocoetes, ventral view.
at., atrium; *cd.* and *cd.'*, cords attaching atrium and ventricle, respectively, to the pericardial walls; *co.*, conus arteriosus; *ij.*, inferior jugular vein; *l.*, liver (anterior tip); *sv*, sinus venosus, *vn.*, ventricle.

The walls of the atrium at diastole (fig. 3) are of almost equal thickness except in the anteromedian angle, where the wall thins out, and at the anterolateral third in the area of the atrial cord (*cd.*), where it is somewhat heavier. In the posterior part just behind the sinuatrial valves, the wall gives the appearance of being heavy as is shown in dotted line, but the lining, which is drawn in solid line in figure 3, shows that it is only of average thickness.

The atrial cord (*cd.*, figs. 2 and 3, and Daniel, 1931) binding the atrium to the pericardial wall near the base of the last branchial arch is made up of a series of nodules with a central strand running through it. In atrial diastole the cord is loose; in systole it is sufficiently taut to exert tension on the pericardial wall. Moreover, in a specimen with the cord severed, the atrium moved backward so that it lay behind instead of at the side of the ventricle.

The aperture between atrium and ventricle is guarded by the atrioventricular valves (*av.*). While the ventricle is filling (fig. 3), these valves are open, and from the atrial side resemble a tent with the curtain opened. When closed (fig. 2), the sides of the tent take the form of parallel wedges, the blunt ends of which are directed toward the atrium, and the funnel-like apex extends into the ventricle.

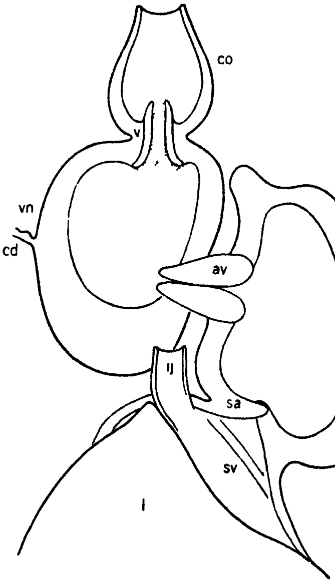

Fig. 2

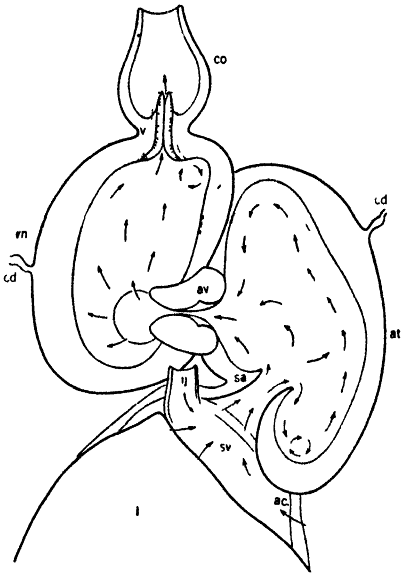

Fig. 3

Fig. 2. Diagram of heart, 10 mm., Ammocoetes in systole.

Fig. 3. Diagram of heart, 10 mm., Ammocoetes in diastole. Arrows indicate direction of flow.

ac., entrance of superior jugular (anterior cardinal) vein; *at.*, atrium; *av.*, atrioventricular valve; *cd.* and *cd.'*, cords attaching atrium and ventricle, respectively, to the pericardial walls; *co.*, conus arteriosus; *ij.*, inferior jugular vein; *l.*, liver (anterior tip); *sa.*, sinuatrial valve; *sv.*, sinus venosus; *v.*, semilunar valve; *vn.*, ventricle.

The ventricle (*vn.*) in general is kidney-shaped, with the hilum or concavity pointing inward at the atrioventricular junction. In diastole the ventricular cavity is much enlarged and the thickness of its walls is difficult to judge accurately, because they are lined with an irregular reticulum into the meshes of which the corpuscles are free to pass. The posterior lateral wall appears compact and thick, but the anteromedian part has one segment which is relatively thin. In systole the ventricular wall is compact and especially thick posteriorly.

A ventricular cord *cd.'* (figs. 2 and 3) similar to that described for the atrium attaches the outer wall of the ventricle to the pericardial wall opposite the position of the atrioventricular valve.

Anteriorly the ventricle joins the bulbus or conus arteriosus (*co.*). The conus has the form of a short-necked *carafe,* the bottom and sides of which are thicker than the neck. The semilunar valves (*v.*) are shown in figures 2 and 3.

THE ACTIVITY OF THE HEART AND THE MOVEMENT OF BLOOD

IN A YOUNG LARVA of from two and a half to three millimeters in length the heart pulsates faintly and at a slow rate; at five millimeters its normal rate has increased to about sixty beats per minute. At this stage a colorless venous flow may be observed to the right and left of the valvular intestine and in the atrium. By the time the animal has reached six to six and a half millimeters the velum begins to beat, the circulation increases in volume, and color begins to appear in the blood corpuscles. The observability of the circulating mass improves greatly following this stage and is at an optimum of visibility up to eleven millimeters.

THE ANAESTHETIZED HEART

IN SPECIMENS of these favorable stages, the atrium and the ventricle are seen to be the most active parts of the heart. The wave of contraction, however, begins near the mouth of the inferior jugular vein, then passes over the sinus venosus, the atrium, and the ventricle, and finally to the conus arteriosus.

Activity in the sinus passes forward to the sinuatrial constriction. The sinuatrial valves (*sa.*, fig. 2) close suddenly, with the result that the blood is carried backward into the mouths of all the entering veins. The instant the mass of blood begins to pass through the sinuatrial aperture, and before the valves are fully opened, the walls of the sinus, observed from the right side, are seen to bulge outward. The valves then float out into the atrium as the blood continues to pass through the aperture.

Blood under venous pressure now pours into the atrium. In its course, a small part of it turns laterally and posteriorly to form an eddy just behind the sinuatrial valve (see arrows, fig. 3); another current flows toward the median line and along the atrial wall to the atrioventricular junction; a third great central column passes obliquely across the atrial cavity and strikes the lateral wall. It then passes either directly toward the atrioventricular junction or first goes to the anterior tip of the atrium and then turns mediad and comes back to the atrioventricular junction. Upon the opening of the atrioventricular aperture, the blood streams through into the ventricle, using the atrium only as a passageway. The atrium then contracts suddenly, forcing all its blood into the ventricle. We may follow this active wave of contraction and the course of the blood more in detail.

As the sinuatrial valves (*sa.*, fig. 2) close, that part of the atrial wall connecting sinuatrial and atrioventricular junctions takes on a vigorous wavelike movement, and the wall just to the side of the sinuatrial junction is thrown into a rocking motion. The synchronous activity of these two posterior areas increases enormously the velocity of the blood which has entered the atrium under venous pressure, so that it is thrown forward and may even reach the anterior tip of the atrium. Instantly the contraction of the distal tip of the atrium meets the oncoming mass and whirls it backward and inward toward the median line and out through the atrioventricular aperture. During this moment of active atrial systole, no part of the atrial wall is free from vigorous activity.

During the time that the atrial blood is passively entering the ventricle, the corpuscles can be seen to spread out fan-shaped among the meshes of the ventricular walls. At this time the ventricle appears to fill completely, but atrial systole now sends a great additional mass of blood into it. The strong atrioventricular valves, which have been open during the passive and active filling of the ventricle, now snap to and stand out as thick parallel wedges. Ventricular systole follows immediately upon atrial systole, the ventricular lumen shortens, and the column of blood is driven ahead, chiefly by the activity of the posterior and lateral ventricular walls. A part of this column enters a *cul de sac* at the anterior and median end of the ventricle and whirls in an eddy before reaching the aperture between ventricle and conus. The main column passes directly forward and out through the conus.

The semilunar valves (*v.*, figs. 2 and 3), guarding this aperture to the conus and closed during the diastole, open suddenly upon ventricular systole, and the mass of blood clears the ventricle and the conus, leaving only a few straggling corpuscles moving slowly forward along the walls of the conus.

ANALYSIS OF THE HEART BEAT

In an anaesthetized heart in which the activity has been reduced to about thirty beats per minute, correlation between the activity in the various parts and structures of the heart and the movement of the blood in its course may be considered in a series of five events or moments, the first of which begins with the entrance of the blood into the atrium and the fifth closes with its exit from the conus.

For the atrium, the first event is initiated by the opening of the aperture and ends with the entrance of the blood into the atrial cavity. At the second moment, the column of blood in the atrium comes almost to a standstill. The atrioventricular aperture opens on the third, and the blood during this and the fourth periods streams through into the ventricle at the same time that it continues to enter the atrium. During the third and fourth moments, the atrium thus, at the same time that it is receiving blood from the sinus, serves as a passageway to the ventricle. The fifth event involves a complicated series of activities, included in atrial systole. In this, the sinuatrial aperture closes suddenly, the atrial walls contract, and all the atrial blood is forced through the atrioventricular aperture into the ventricle.

During the first moment in which the blood is entering the atrium, the ventricle is completing or has completed systole; during the second, when there is quiet in the atrium, the ventricle is empty. Early in the third event, the atrioventricular aperture opens and blood begins to pour into the ventricle. This continues to enter passively from the atrium during the third and fourth moments. At the early part of the fifth, a great column of blood is shunted into the ventricle from the atrium, which at the end of this period is forced out by ventricular systole.

The behavior of the valves of the heart at these different moments is as follows: The sinuatrial valves open at the beginning of the first event and remain open during events two, three, and four, while the blood is passing through the sinus. Early in event five the sinuatrial valves close and the blood in the sinus is forced back into the veins, and that in the atrium, into the ventricle. The atrioventricular valves are closed during events one and two; at three they open, and remain open on count four. At the end of event five, the valves close with the atrium in systole and the ventricle in diastole.

The Dying Heart

The anaesthetized heart may be retarded to a slow rate without apparent ill effects. But when conditions come to be highly unfavorable, as, for example, by the evaporation of the water in which the animal is kept, then the heart begins to show symptoms of distress While this distress may manifest itself in several different ways, the most common form is that of irregularity in the ventricular beat.

The dying ventricle first shows a tendency to block or skip beats. In the earliest form of this behavior, there may be one ventricular beat to every two or three atrial pulsations. By the third atrial systole, the ventricle is gorged with blood, and usually by this time ventricular systole takes place. If this does not occur, after the third atrial systole a small amount of blood passes in and out through the atrioventricular aperture with atrial activity. The degree of ventricular irregularity may be shown in the following table:

TABLE 1

To Each Ventricular Systole the Following Number of Atrial Beats, Dying Heart of Ammocoetes

(10 mm.)

3	10	1	5	3	8	1	3	7	3
3	5	7	8	1	3	3	2	7	3
5	2	2	1	2	2	4	2	3	2
16	11	3	16	4	3	2	15	12	10
5	11	11	2	2	4	4	4	5	18

The most impressive characteristic of the dying heart is the faithfulness of the atrium. During the ventricular distress which we have discussed, the atrial beat may be relatively regular and this regularity may continue long after the ventricle is quiet. In time, however, the atrium itself decreases in tone and the beats become feeble and irregular. The distal part of the atrium generally becomes inactive first, while the parts of the atrial wall behind the sinuatrial valve and between the sinuatrial and atrioventricular junctions, together with the sinuatrial valves themselves, are still active. At this stage the atrium may be filled with blood. A little later the valves may stop, whereupon blood passes in and out through the sinuatrial aperture. In a later stage the atrium may continue to beat feebly with no blood remaining in its cavity.

The sinus venosus may beat with the regularity of the atrium or with two regular pulsations to one of the atrium. The first occurrence which I observed of this double number of pulsations was in a heart in which the ventricle was alive and the atrium regular but slow. There were two distinct beats in the sinus to one of the atrium. In a second specimen I observed two pulsations in the inferior jugular vein to one partial closure of the sinuatrial valve.

In other specimens I observed that the atrium might continue to beat after all activity had ceased both in the sinus and at the base of the inferior jugular. In one which I may cite finally, the last sign of life to remain was a distinct and regular beat at the base of the inferior jugular vein on the side wall where it joins the liver.

Revival of the Heart Beat

After every part of the heart has stopped and the blood can be seen to be laked in various parts of the body, add but a quarter of a drop of water to the organism and the whole wonderful mechanism starts up again. The velum begins to draw in the respiratory current. The corpuscles, which may have lost their haemoglobin, enter into the active circulation, and the organism is virtually recalled from death.

THE VENTRAL AORTA

Aorte ascendante—Milne-Edwards, 1858.
Artiere branchiale—Milne-Edwards, 1858.
Artiere branchiale primare—Julin, 1887.
Arteria branchiale—Carazzi, 1905.
Arteria branchialis—Müller, 1839; Dohrn, 1888.
Branchial artery—Monro, 1785; Owen, 1866; Balfour, 1878; Allen, 1905.
Cardiac aorta—Huxley, 1871.
Haupstamm der Kiemenarterie—Wiedersheim, 1886.
Kiemenarterienstamm—Stannius, 1846, 1854.
Truncus arteriosus—Hochstetter, 1906.
Ventral aorta—Balfour, 1878; T. J. Parker, 1886; Daniel, 1922, 1928, 1934; Corrington, 1930.

THE VENTRAL AORTA (*va.*, fig. 4) in Ammocoetes continues forward from the conus arteriosus throughout the pharyngeal area. Through the first four segments of its course, it is an unpaired vessel; through the remaining four segments, it is paired.

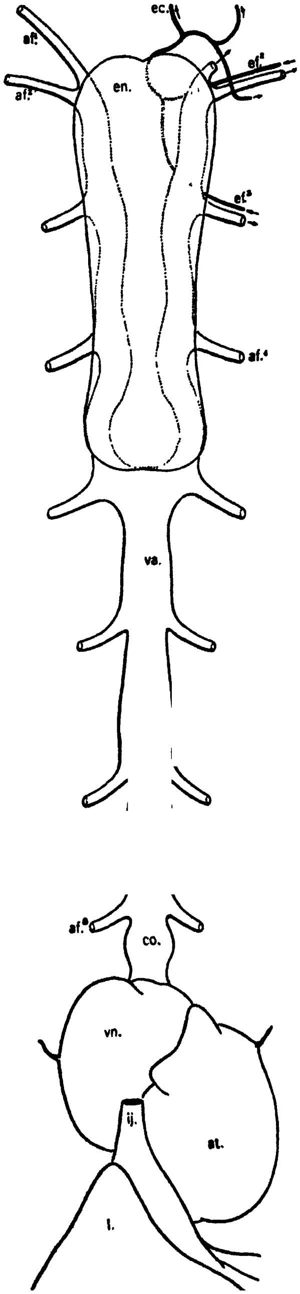

Fig. 4. Ventral aorta and afferent arteries, 10 mm., Ammocoetes, ventral view. *af.*$^{1-8}$, first and eighth afferent arteries, respectively; *at.*, atrium; *co.*, conus arteriosus; *ec.*, external carotid artery; *ef.*$^{2-3}$, second and third efferent arteries, respectively; *en.*, endostyle; *ij.*, inferior jugular vein; *l.*, liver; *va.*, ventral aorta; *vn.*, ventricle.

In general plan the ventral aorta is like that in the Elasmobranch fishes (Daniel, 1934). Both in the Elasmobranchs and in Ammocoetes, the division of the unpaired into the paired part takes place where it approaches or comes into contact with the posterior part of the thyroid gland (endostyle in Ammocoetes). In the Elasmobranchs, the thyroid is limited to one or at most to two anterior segments; but in Ammocoetes, the endostyle is of unusual length, extending through four metameres or body segments and posteriorly well into the pharynx (see *en.,* fig. 5). The divisions of the ventral aorta at the posterior part of the endostyle are located to the right and left and gradually rise until they lie in dorsolateral grooves along the endostyle. They next extend in these grooves to the anterior tip of this organ and are then continued forward toward the head by the first afferent arteries (*af.*^{1}, figs. 4 and 9).

The course of blood through the unpaired and paired parts of the ventral aorta can be followed in detail in a ventral view of a seven- to a ten-millimeter larva. In fact, the circulation may be followed later up to a stage in which pigment invades and obscures the ventral part of the pharynx. Following immediately upon its course through the bulbus or conus arteriosus, the blood passes along the unpaired vessel and at its bifurcation curves outward around the posterior part of the endostyle and mounts in the paired vessels to the dorsolateral grooves along which the main currents of blood jet forward.

From the dying heart or from the heart which has been inhibited by drugs, it can be seen that the blood in the ventral aorta is driven forward chiefly by ventricular contraction. The head of the column, while lagging a bit, goes forward with only a slight decrement in rate even at the anterior part of the paired division.

THE AFFERENT BRANCHIAL ARTERIES

Afferent Branchial Artery—T. J. Parker, 1886; Allen, 1905; Allis, 1908; Daniel, 1922, 1928, 1934; Corrington, 1930.
Artère branchiale propre—Milne-Edwards, 1858.
Arteria branchialis—Hyrtl, 1872.
Arterie afferenti—Carazzi, 1905.
Branch of branchial artery—Monro, 1785.
Kiemenarterie—Müller, 1839; Stannius, 1846, 1854; Hyrtl, 1858, 1872; Dohrn, 1888.

IN THE REGION OF THE PHARYNX, the ventral aorta gives off seven pairs of afferent arteries (*af.*$^{2-8}$, fig. 4) and its extension forward into the first gills on the left and right sides respectively may be properly regarded as another pair, the first afferent arteries(*af.*^{1}). Of these eight pairs of afferents, the eighth to the sixth leave the unpaired trunk. The fifth leaves the ventral aorta near its bifurcation and may arise from the unpaired trunk, although it usually comes off from the paired ventral aorta just around the posterior angle of the endostyle. The fourth to the second afferents spring from the paired ventral aorta, and the first, as we have said, continues it forward.

The eighth or most posterior afferent (*af.*^{8}) is relatively small, even where it leaves the ventral aorta. It passes over the ventral cartilage and out into the

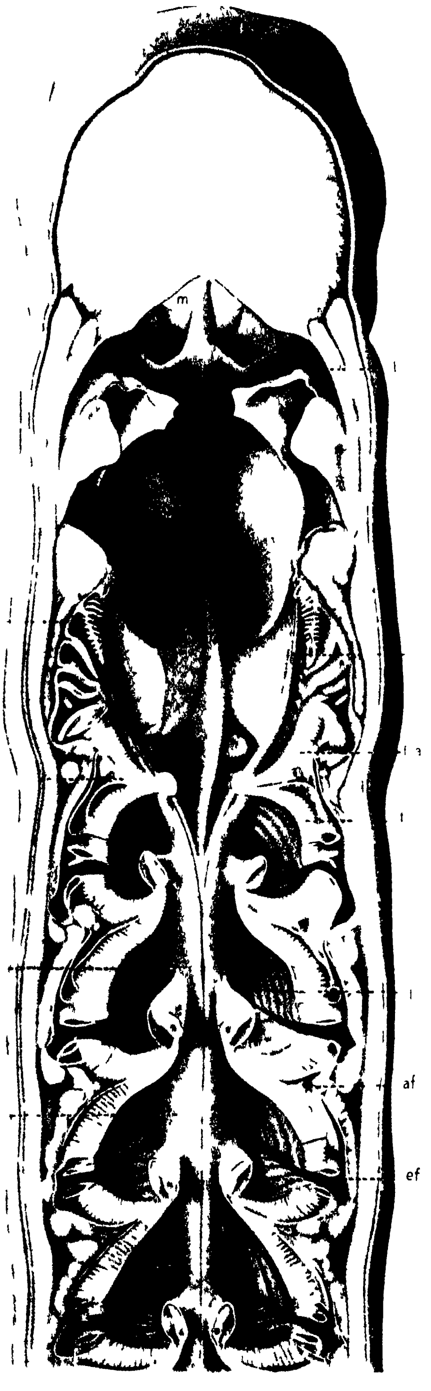

Fig. 5. Head and upper part of pharynx with roof removed, older larva, Ammocoetes, dorsal view.

af., afferent artery; *bc.*, buccal cavity; *en.*, aperture from endostyle, *ef.*, efferent artery; *en.*, endostyle; *fl.a.*, *fl.p.*, anterior and posterior filaments, respectively, of holobranch; *gp.*, gill pouch; *m.*, mouth; *pg.*, peripharyngeal grooves, *s.*, septum; *vl.*, velum.

septum which bears the gills, taking up its position in the outer edge of the septum (for plan, see *s.*, fig 6). In this position at the attachment of the septum, it increases to a sinus-like vessel which extends above the septum almost to the middorsal line.

The column of blood in this vessel can be observed to leave the ventral aorta, pass over the cartilage, and then along the septum not far from the surface. As it flows along the septum, it gives out blood to anterior gills. By the time it has reached the dorsalmost part of the septum, the column has dwindled to small size.

Other afferents from the seventh to the second take up their position in the attached margins of the septa similar to the eighth (see *af.*, fig. 5). These afferents differ from the eighth, however, in one important respect, that is, along the septa they give off alternate afferent arterioles anteriorly and posteriorly to gills located on the anterior and posterior surfaces of the septa, respectively.

The second afferent (*af.*^{2}, figs 4 and 9) may be described more in detail since it is typical of the whole series and the course of the blood in it is more completely observable than that of any of the others. At its union with the paired ventral aorta near the anterior tip of the endostyle, it is a relatively much stronger trunk than is the eighth afferent. Like the eighth, however, it extends out over the cartilage (*bc.*), which has been cut in figure 9, to the septum and then up the septal attachment dorsally. Along its course it gives off some fifteen afferent arterioles to the gills. Usually eight of these supply anterior gills on the septum and seven, posterior gills. At its dorsal tip, after having given off these branches, it bends backward so that the tip if seen from an anterolateral view resembles the handle of a walking-stick.

At its origin the second afferent carries a large column of blood which as it rises can be seen to flow into the gills and temporarily disappear. If observed in dorsolateral view and seen from in front, the column of blood, even in the dorsal segment of this vessel, is still of considerable size and the method of its entrance into the last gill near the dorsal attachment of the septum behind the otic capsule may be followed.

The first afferent (*af.*^{1}, figs. 4 and 9), with its circulation, calls for special consideration. In order to understand its position, it is necessary to say that the place of attachment of the first septum ventrally is considerably posterior to that of its dorsal attachment so that the septum on which the artery is located is directed more obliquely forward than are succeeding septa. As the first afferent rises in the septum, it carries a large column of blood which is distributed to the relatively long gills located only on the posterior surface of the septum.

AFFERENT AND EFFERENT ARTERIOLES AND BRANCHIAL CIRCULATION

Afferent branchial arteriole—Daniel, 1922, 1928, 1934; Corrington, 1930.
Afferent filament artery—Allen, 1905.
Artériole—Milne-Edwards, 1858.
Ast der Kiemenarterie—Müller, 1839.
Branch of the afferent artery—T. J. Parker, 1886.
Efferent branchial arteriole—Daniel, 1922, 1928, 1934; Corrington, 1930.
Ast der Kiemenvene—Müller, 1839.
Branch of the efferent arteries—T. J. Parker, 1886.
Efferent filament artery—Allen, 1905.

In a ventral or a dorsal view of a ten-millimeter Ammocoetes, circulation in the individual gill can be readily observed. The base of the gill (*g.*, fig. 9) is broad and is so attached to the septum as to run somewhat obliquely to the course of the afferent artery (*af.*^{2}). The afferent arteriole which is to supply the gill does not arise where the afferent first reaches the base of the gill, but it comes off from the afferent at the opposite side of the base of the gill. From this point it takes up its position along the margin of the gill and extends down to the opposite side, where it connects with its efferent arteriole. From here the vessel joins the efferent artery (*ef.*^{2}) which, as we shall see later, lies along the inner or *free* margin of the septum.

A diagram (fig. 6) will make the course of the blood through the gill clear. The gill at its base is oblique to the afferent artery (*af.*^{2}) and receives the blood from the afferent arteriole (*a.*) near its distal margin. The blood passes through the loop in which it is oxygenated and down the proximal side of the filament through the efferent arteriole (*b.*), and finally out through the efferent artery (*ef.*^{2}).

THE EFFERENT BRANCHIAL ARTERIES

Efferent branchial artery—Daniel, 1922, 1928, 1934; Corrington, 1930.
Aortenwurzel—Hyrtl, 1858, 1872.
Artère épibranchiale—Milne-Edwards (in part), 1858.
Arteria efferente—Carazzi (in part), 1905.
Branchial vein—Monro, 1785; Owen, 1866; Huxley, 1871; Balfour, 1878.
Epibranchial artery—T. J. Parker, 1886; Allen, 1905; O'Donoghue, 1928.
Epibranchial portion—Allis, 1908.
Kiemenvene—Stannius, 1846, 1854; Hyrtl, 1858; Dohrn, 1888.
Radix aortae—Wiedersheim, 1886.
Veine branchiale*—Milne-Edwards (in part), 1858.
Vena branchialis communis—Müller, 1839.

The first efferent (*ef.*^{1}, fig. 9), unlike the remaining seven, curves forward dorsally and under the ear capsule. The second to eighth remaining efferents are attached to the dorsal aorta and are so deep at this position that they are difficult to observe. All the efferents are relatively easily seen, however, in side view (*ef.*^{2}, fig. 9) where, as we have said, they are located along the free edges of the septa. Where they originate ventrally, that is, where they are joined by the first efferent arterioles from the gills, they are small. Dorsally, they increase in caliber until finally where they join the dorsal aorta they are of relatively large diameter.

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* Milne-Edwards, vol. 3, p. 334, explains the term branchial vein as meaning a vessel like a pulmonary vein, bearing oxygenated blood.

The corpuscles can be clearly seen as they pass around the margin of a gill and down the side opposite to that from which they entered, but their approach to the efferents is difficult to observe. In certain places, however, the intervening tissues are clear and the corpuscles may be seen as they enter the efferents. As the column of blood passes upward in the efferent arteries, especially if it receive blood both anteriorly and posteriorly, it increases greatly in size. This may be made out best where the circulation is slow and each pulsation seems strained to lift the weight of blood.

Two important branches are associated with the first and second efferent arteries. These are a dorsal vessel, the orbital artery (*oa.*, figs. 9 and 11) from the first efferent to the area around the eye and the roof of the oral hood, and a ventral artery, the external carotid (*ec.*, figs. 4 and 6) from the second efferent to the lower jaw. We shall consider the latter first.

THE EXTERNAL CAROTID ARTERY

Afferent mandibular artery—Allis, 1908.
Anterior hyal efferent—Allis (in part), 1908.
Arteria mandibulare—Carazzi, 1905.
Arteria submentalis—Hyrtl, 1872.
Arteria thyreo-maxillaris —Hyrtl, 1872.
Carotis externa—Julin, 1887; Dohrn, 1888.
External carotid artery—De Beer, 1924; O'Donoghue, 1928.
External lateral hypobranchial artery—Allis (in part), 1908.
Hyoideomandibular artery—Daniel, 1922, 1928.
Hyomandibular artery—Corrington, 1930.
Mandibular artery—T. J. Parker, 1886; Ferguson, 1911.

IN AMMOCOETES the external carotid (*ec.*, figs. 4 and 6) arises as the ventral extension of the second and the third efferent arteries. As the corpuscles leave the most ventral gills of these arches, they pass down over the endostyle (fig. 4) bearing oxygenated blood. The ventral extension of the third efferent (*ef.*^{3}) then bends forward to join the second and the common trunk as the external carotid (*ec.*, figs. 4, 6) curves slightly outward at the anterior end of the endostyle. This gives off a branch laterally which in turn divides into an anterior branch (*v.*, fig. 6) to the ventral part of the velum, and a posterior artery that passes over and up around the posterior side of the second arch and then backward, carrying blood away from the external carotid instead of to it, as described by Dohrn (1888). The external carotid now curves back toward the midventral line and runs forward to supply the lower lip. Near the lip it divides more or less irregularly into a median and a lateral branch. The lateral branch extends upward along the lower lip to the notch and then upward and backward to appear on the side (*ec.*^{1}, fig. 9). We shall return later to a consideration of this interesting condition.

THE ORBITAL ARTERY

Arteria carotis externa—Stannius, 1854.
Artère faciale—Milne-Edwards, 1858.
Arteria orbitale—Carazzi, 1905.
Arteria orbitalis—De Beer, 1924.
Carotid—Owen, 1866.
Carotide interne postérieure—Milne-Edwards, 1858.
Carotis externa—Hyrtl, 1858, 1872.
Carotis interna posterior—Müller, 1839.
Carotis posterior—Müller, 1839; Stannius, 1846, 1854.
External carotid artery—Allen, 1905; Allis, 1908; Daniel, 1922, 1928; Corrington, 1930.
Posterior carotid—T. J. Parker (in part), 1886.
Stapedial artery—O'Donoghue, 1928.

AFTER THE FIRST EFFERENT ARTERY has passed under the ear capsule (*ef.*^{1}, figs. 9 and 11), it joins the paired dorsal aorta (*da.*^{1}, fig. 11). The common stem produced by this union, which I have assumed is the internal carotid, extends forward toward the eye. The internal carotid (*i.c.*, fig. 11) then passes inward to the midventral line, and the orbital artery (*oa.*, figs. 9 and 11) swings outward toward the orbit. The orbital supplies the muscles of the eye and then runs forward. After it leaves the orbit, it gives off a ventral branch which curves backward under the eye to supply the dorsal part of the velum (*vl.*, fig. 9). A second branch curves ventrally in the area of the olfactory lobes (*ol.*) and the main artery then sends branches forward over the tip of the oral hood.

RELATION OF ORBITAL ARTERY TO EXTERNAL CAROTID

In the first and second editions of my book on the Elasmobranch Fishes (Daniel, 1922 and 1928), I followed Hyrtl (1858) in the use of the term external carotid (*carotis externa*) as applied to the artery which runs through the orbit and on to the rostrum. O'Donoghue (1928), however, has since made it reasonably certain that the external carotid is an artery belonging primarily to the lower jaw. I am therefore here designating the artery of the orbit, the stapedial artery in Amniota, as the orbital artery.

The relation of the orbital (stapedial) to the external carotid in man is highly interesting (Tandler, 1902; Evans, 1912). In the human embryo these two systems anastomose, but during development the stapedial stem breaks at its place of origin, thus giving in the adult the anomalous condition of the area around the eye supplied by the external carotid, which belongs to the region of the lower jaw. In some other mammals, as, for example, the rat, these two systems are bound together by a strong anastomosis, but the stapedial does not break at its place of origin and hence the two systems, stapedial (orbital) and external carotid, supply the areas around the eye and the lower jaw, respectively. In the Elasmobranch fishes, the orbital supplies the muscles of the eye and a branch given off from it in the posteroventral part of the orbit *may* anastomose with the branch coming up the hyoid from the external carotid.

After the orbital has left the orbit anteriorly, it divides much as it does in Ammocoetes.

Through O'Donoghue's work we now have a satisfactory history of these vessels in all Gnathostomes. I should like here to trace the history still further back to the Cyclostomata.

In Ammocoetes the lateral branch of the external carotid (*ec.*[1], fig. 9) on each side, as we have seen (p. 325), passes upward and appears superficially and laterally where it breaks up into capillaries to the posterior and lower part of the oral hood. The main stem of the orbital artery likewise breaks up into a net on the anterodorsal part of the hood, and these two capillary nets converge to form a large facial vein (*fv.*, fig. 9) which carries the blood to the hyoidean sinus (*hs.*). The ventral branch of the orbital (*vo.*, fig. 9) also contributes in part to this capillary net. In part, it supplies a branch (*v.*[1]) to the dorsal side of the velum (*vl.*), which drains by its own vein (*dv.*, fig. 9) to the same sinus.

In a dissection of the blood system of higher types, only the grosser vessels appear; but in a study of the circulation in a semitransparent living animal like Ammocoetes, no part of the picture escapes direct observation. Dynamic and significant changes in the course of the blood and complete shifting of relations and allegiance of vessels to systems may be observed to occur within the brief span that it takes for an animal to increase a single millimeter in length. Thus the early passageway for the corpuscles as they reach the end of the facial vein (*fv.*) is upward over the otic capsule anteriorly to join the superficial jugular vein (*j.*, fig. 9). A short time later, all the corpuscles from the facial vein move across in the upper part of the hyoidean sinus (*hs.*) under the otic capsule to the superior jugular (*sj.*) behind the ear. Similarly the vein terminating the newly formed ventral orbital artery may at first connect with the superficial jugular vein (*j.*, fig. 9) in front of the ear capsule; and the corpuscles may pass temporarily along that course and on back by way of the superior jugular (*sj.*) to the heart. Soon, however, the mass of blood increases in the ventral orbital vein, and it then (*ov.*, fig. 9) presses down into the dorsal tip of the hyoidean sinus like a cone, thus diverting the corpuscles which are entering the sinus from the facial vein so that in a ten-millimeter larva the column in the facial is also forced downward, and its course from now on is first ventral and then upward and behind the island in the hyoid and finally obliquely posterior and under the otic capsule to enter the superior jugular on its median side. The blood from the ventral orbital vein usually passes directly backward above the island, but it may leave the sinus with the blood from the facial or it may pass into the superficial system and return to the heart either by a dorsal or by a ventral route.

THE HYOIDEAN SINUS

THE HYOIDEAN SINUS (*hs.*, figs. 8 and 9) in a ten-millimeter Ammocoetes is a vessel of large caliber, being wide at the dorsal end and narrowing ventrally almost to the midventral line. Into the dorsal part of the sinus anteriorly enter the ventral orbital vein (*ov.*, fig. 9) and the facial vein (*fv.*) which we have discussed, and the dorsal velar vein (*dv.*). At the posterior upper angle, the hyoidean sinus runs under the otic capsule obliquely backward to join the superior jugular vein (*sj.*) on its median side. Just below the posterodorsal angle of the sinus where it leads to the superior jugular vein, the sinus is connected with a longitudinal and superficial vein (*ls.*) which extends across the first and second arches, receiving from these two arches a segmental vein before it joins the superior jugular midway between the second and third arches.

If the animal studied be in a normal position with the back upward, the mass of blood in the large hyoidean sinus may move upward and backward under the ear capsule and on to the heart through the superior jugular. But the blood is free to pass downward along the sinus. If it does this, when it reaches the ventral angle of the sinus, it enters the inferior jugular vein (*ij.'*, fig. 8). Into the lower and narrower end of the hyoid sinus, a ventral velar vein (*vv.*) also joins it. At its ventral tip, where the sinus joins the inferior jugular, it receives from the midventral lines two parallel vessels. The anterior of these is formed by a midventral vessel from the lower lip, and the posterior, from a similar vein on the midventral line of the endostyle.

ENDOSTYLAR CIRCULATION

I HAVE BEEN SUCCESSFUL in observing the entrance of arterial blood to the endostyle only in front of the fourth afferent artery. The circulation in the capillaries and veins of the endostyle provides a remarkable and fascinating spectacle which is but poorly represented in figure 7. The vessels for circulation in the two sides of the gland vary somewhat in their actual pattern. Great difference also obtains in the circulation itself, depending upon whether the animal is tilted toward the left or the right side. In ventral view as one looks down on the endostyle, the first impression is that of whirlpools of corpuscles appearing suddenly through tiny openings between the columns of secreting cells and running in tiny vessels that take spiral courses. In some of these, the corpuscles whirl toward the midline; in others, away from it. In the latter the corpuscles may pass into the inferior jugular by the segmentally arranged vessels; in the former, they enter the midventral, endostylar vein and pass either forward or backward, depending on their location in the endostyle. Whichever course they take, they enter the right or left inferior jugular on their way back to the heart.

In a specimen with the right side turned slightly upward, I observed a striking phenomenon. In the spiral just anterior to the segment of the fourth afferent artery, the corpuscles coursed through the narrow vessels for a moment as a rapidly whirling mass. The circulation then ceased for a time and later began whirling again. Upon closer observation, I noticed that the aperture through which the corpuscles entered had a valvular action forcing them out in rapid succession across the median column of the endostyle to the midventral vein. In this they surged forward as a dense mass and turned laterally around the tip of the endostyle to the ventral tip of the hyoid sinus (*hs.*, fig.

8). From this position, they were free to go up the hyoid sinus to the superior jugular system, or to enter the inferior jugular and go back to the heart by that route. In the period of rest when the aperture was not actively opening and closing, the corpuscles flowed leisurely across the median column to the median vein and dropped back posteriorly, then laterally to the inferior jugular.

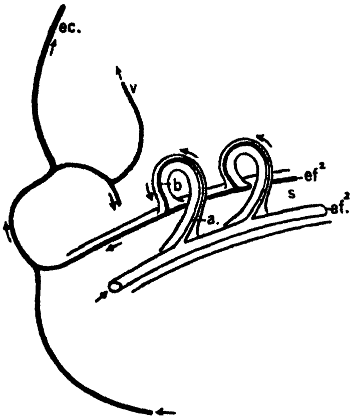


Fig. 6. Diagram of branchial circulation and origin of external carotid artery. *a.*, afferent arteriole; *af.*², second afferent artery; *b.*, efferent arteriole; *ec.*, external carotid artery; *ef.*², second efferent artery: *s.*, septum of gill; *v.*, ventral velar artery.

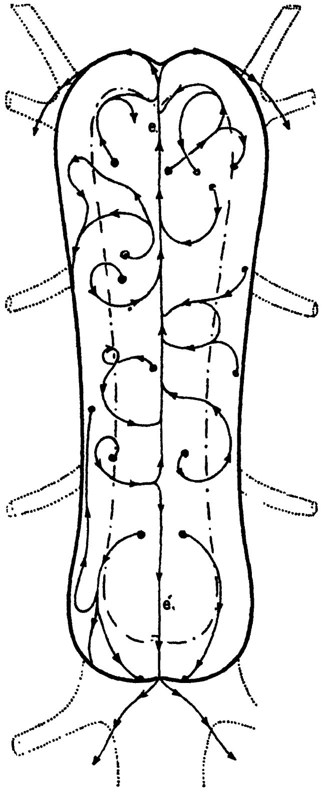


Fig. 7. Circulation in endostyle, 10 mm., Ammocoetes, ventral view. Course of corpuscles indicated by arrows. *e.*, anterior endostylar vein; *e.'*, posterior endostylar vein.

THE INFERIOR JUGULAR VEIN

A small vein somewhat like to our anterior external jugular—Monro, 1785.
Vena jugularis inferior—Müller, 1839; Stannius, 1846, 1854.

The inferior jugulars (*ij.'*, fig. 8) are paired vessels which reach from the anterior segment of the endostyle back to the heart. At the heart, right and left jugulars unite into a common trunk (*ij.*, figs. 1, 2, 3, 4, and 8). In its course back to the heart, each of the paired jugulars receives laterally nutrient or segmented veins (*sg.*, fig. 8). Each also receives on its median side segmentally arranged veins from the endostyle previously mentioned.

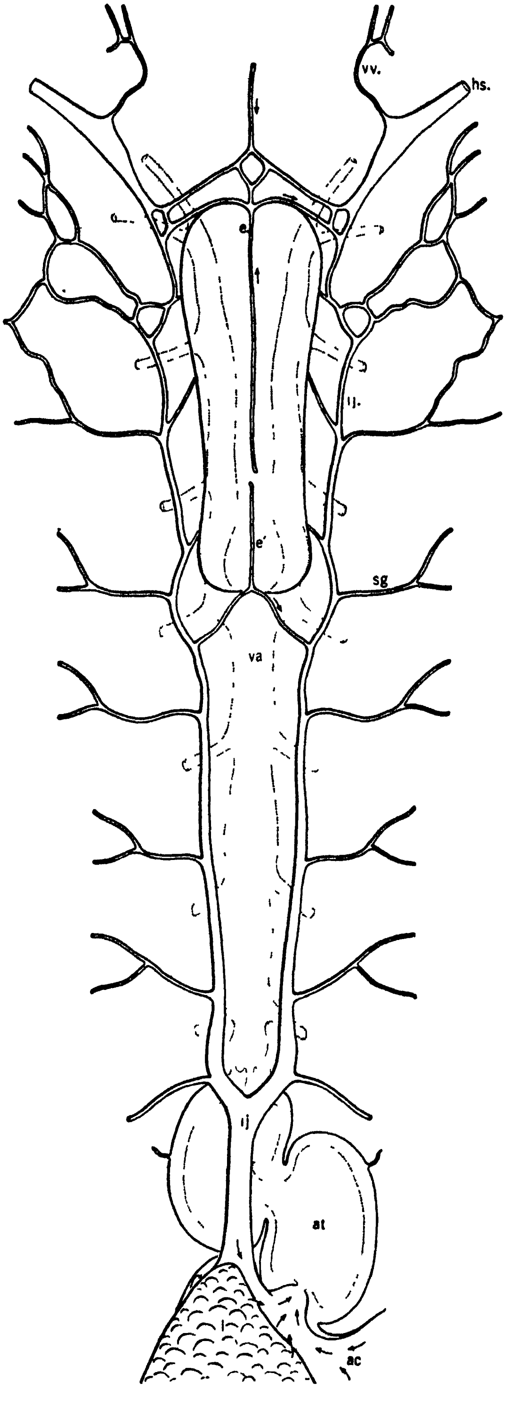
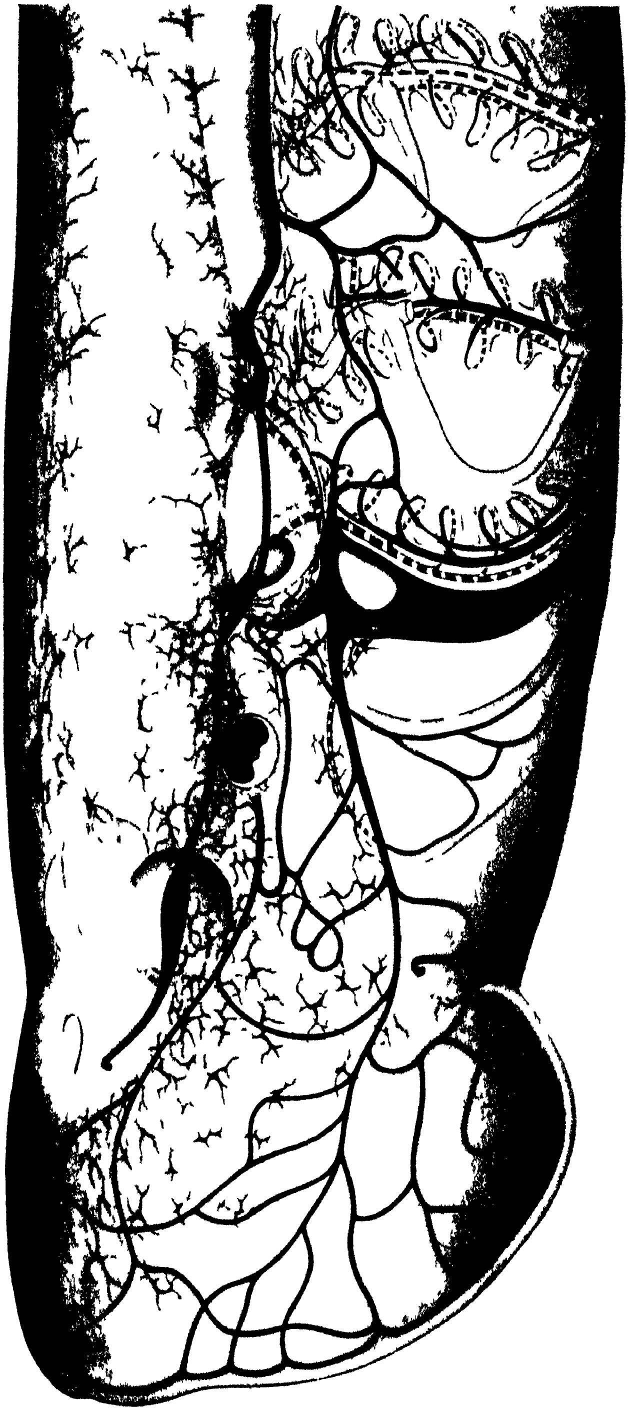


Fig. 8. Inferior jugular veins and their tributaries, Ammocoetes.

ac., entrance of superior jugular (anterior cardinal) vein to sinus venosus; *at.*, atrium; *e.*, anterior median endostylar vein; *e.'*, posterior median endostylar vein; *hs.*, ventral part of hyoidean sinus; *ij.*, unpaired inferior jugular vein; *ij.'*, paired inferior jugular veins; *l.*, liver; *sg.*, segmental vein to the inferior jugular; *va.*, ventral aorta; *vv.*, ventral velar vein.



Where the unpaired jugular enters the sinus venosus, it is a vessel of large size. The great column of blood collected along its course pours into the heart as a steady stream checked only at the closure of the sinuatrial valves. In the dying heart, I have observed certain changes in the normal (passive) behavior of the inferior jugular, some of which I have already recorded (p. 318). Others I here report.

In one specimen in which the ventricle was quiet and the atrium active, there was a back effect in the inferior jugulars which reached as far forward as the fourth afferent artery. All tributaries of the inferior jugular at this time were gorged with blood. In another specimen, ventricular and atrial beats were slow but fairly regular. The base of the inferior jugular, as well as the sinus venosus, underwent a pulsation of two clear beats to a single one of the atrium and one of the ventricle. The pulsations in the inferior jugular then ceased and the atrium became feeble. Revived with water, the atrium and the ventricle drew out almost in a straight line; the inferior jugular and the sinus were active, but the atrium and the ventricle were halting in their activity although equal in number of pulsations. The column of blood in the inferior jugular moved back and forth twice to each closure of the sinuatrial valves. A final observation already recorded may be repeated here. In this specimen the last sign of life was a distinct and regular beat at the base of the inferior jugular vein.

THE INTERNAL CAROTID ARTERY

Arteria carotis interna—Stannius, 1846, 1854.
Artère carotide interne—Milne-Edwards, 1858; Julin, 1887.
Carotide posteriore—Carazzi, 1905.
Carotis anterior—Müller, 1839; Stannius, 1846, 1854.
Carotis interna—Hyrtl, 1858, 1872.
Carotis interna anterior—Müller, 1839.
Internal carotid artery—Allen, 1905; Allis, 1908; Daniel, 1922, 1928, 1934.
Paired dorsal aorta, pseudobranchial sections—Corrington, 1930.
Part of posterior carotid plus commissure "w"—T. J. Parker, 1886.

THE INTERNAL CAROTID (*ic.*, fig. 11), which continues the paired dorsal aorta (*da.'*) forward and inward, after having given off the orbital artery (*oa.*) turns in toward the brain and runs so deep that it can only be followed in an occasional favorable specimen. In figure 11 its branches and relationships are shown. As it passes inward, it approaches or joins a similar artery from the opposite side and then curves outward and forward around the infundibulum, giving off a branch which passes to the anterior part of the nasal apparatus. The main artery then turns upward between the olfactory and optic lobes as the cerebral artery (*ca.*).

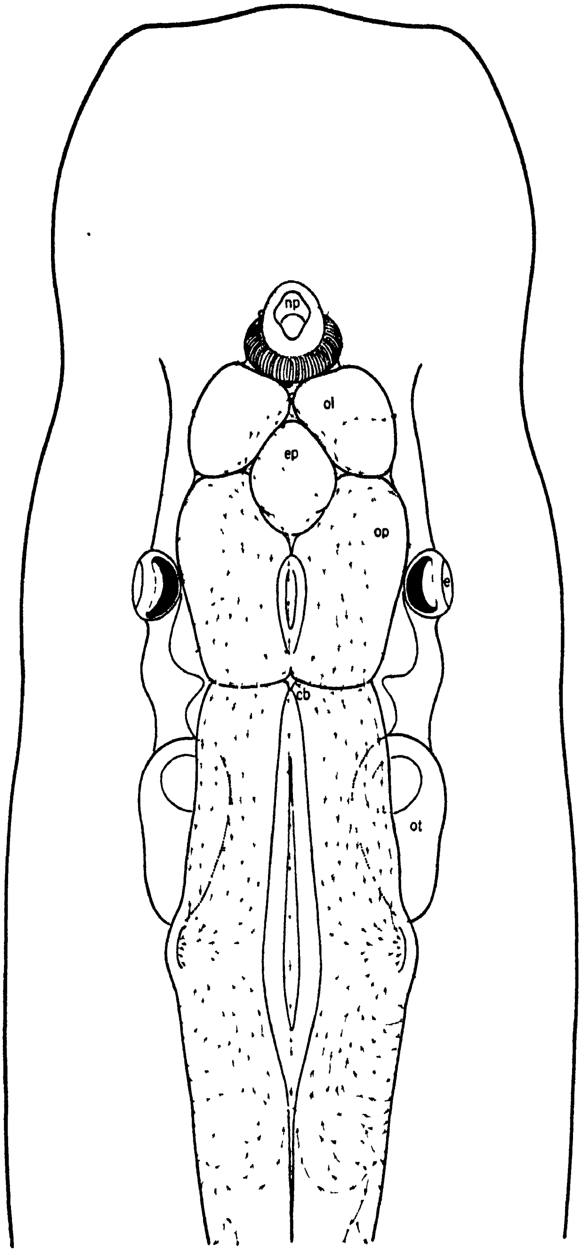


Fig. 10. Circulation on brain, 10 mm., Ammocoetes, dorsal view. Source and direction of flow shown by arrows. Light blue in great dorsal sinus; orange in superficial jugular vein.

cb., cerebellum; *e.*, eye; *ep.*, pineal body; *np.*, nasal pit or aperture; *ol.*, olfactory lobe; *op.*, optic lobes; *ot.*, otic or ear capsule.

A great column of blood is carried by the internal carotid artery. I have observed in a number of specimens which had little pigment that the blood in this vessel comes not only from the first efferent artery above but also, in part, from the second efferent. The blood from the second efferent as it pours into the unpaired dorsal aorta divides into two currents. Part of it flows backward in the aorta toward the body and part forward toward the head. At the anterior third of the otic capsule, the forward current separates and passes left and right through the paired dorsal aortae (*da.′*, fig. 11). At the opening of the first efferent (*ef.*^{1}), the circulating mass increases its volume. About one-third of the corpuscles are next shunted outward through the orbital artery (*oa.*) and those remaining form a broad column which passes by way of the internal carotid (*i.c.*) inward and around the anterior tip of the notochord (*ch.*). These corpuscles (-|-|→, fig. 10) reappear in large part, as we shall see in a moment, as they enter the sinuses over the brain.

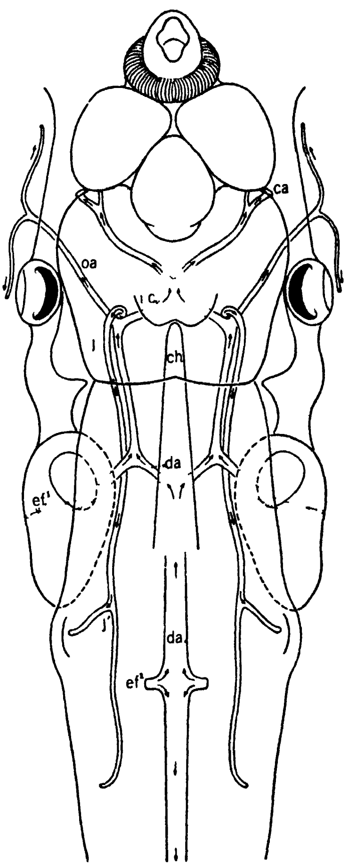


Fig. 11. Internal carotid artery and its relations, 10 mm., Ammocoetes.

ca., cerebral artery; *ch.*, notochord; *da.*, unpaired dorsal aorta; *da.′*, paired dorsal aorta; *ef.*$^{1-2}$, first and second efferent arteries, respectively; *i.c.*, internal carotid artery; *j.′*, deep jugular vein; *oa.*, orbital artery.

CIRCULATION OVER THE BRAIN

The movement of blood over the brain of Ammocoetes provides a rare spectacle. This spectacle is produced in part by the fact that the blood moves in immense cavities and consequently that millions of corpuscles pass by in a single view (see fig. 10). Again, if the body be tilted to the right or left, this mass effect is augmented, for the floating hordes of cells may be found flowing on one side only, while the other side may be free from blood for long periods of time.

THE DORSAL SINUS

The volume of circulation of the brain takes place in this overlying sinus. The most anterior vessel to enter it comes up between the olfactory lobes (*ol.*, fig. 10), and the corpuscles from this, represented by arrows with solid heads, flow backward around the pineal body (*ep.*) and over the optic lobes (*op.*) toward the median line. At this position they are joined by the many corpuscles (-|-|→) which come up from the cerebral artery (*ca.*, fig. 11) between the optic and olfactory lobes; and these combined streams spread out in the form of a broad band as it passes backward over the optic lobes (*op.*). If the specimen studied be tilted slightly to right or left, many of the corpuscles will flow across to the opposite side in the groove separating optic lobes and cerebellum (*cb.*); otherwise the broad column of corpuscles passes on back over the otic capsule (*ot.*), where it meets a counter current coming forward from the roof of the medulla. The two counter currents clash behind the otic capsule, swerve outward, and pour down as a great funnel (*fl.*, fig. 9), the posterior cerebral vein (*pc.*, figs. 9 and 10), and into the superior jugular vein.

The relation of the venous blood to the brain in Ammocoetes is unlike that in the Elasmobranch fishes. In the Elasmobranchs well formed posterior cerebral veins (Rex, 1891; O'Donoghue, 1914, 1928; Daniel, 1922, 1928, 1934) relieve the blood from the optic lobes in part, and from the cerebellum and medulla, and carry it back to the anterior cardinal sinus. In Ammocoetes the only part of this posterior cerebral vein (*pc.*, fig. 9) present is the terminal funnel by which it leaves the sinus. The funnel receives as well all venous blood from the anterior parts of the brain, and consequently an anterior cerebral vein in Ammocoetes is absent.

THE SUPERFICIAL AND DEEP JUGULAR VEINS

Veine jugulaire superficielle—Julin, 1887.
Veine jugulaire profonde—Julin, 1887.

THE SUPERFICIAL JUGULAR (*j.*, fig. 9) reaches from the nasal apparatus to the posterior end of the otic capsule and runs superficially over the eye and the ear capsule. Through an asymmetrical aperture on one side of the anterior part of the nasal pit, corpuscles enter this vein and some of these flow forward around the nasal apparatus and pass backward in the vein on the opposite side. Others enter posterior to the nasal apparatus. These two streams unite and move backward in a wider sinus over the olfactory lobes (*ol.*, figs. 9 and 10) and then swing outward at the optic lobes and around the otic capsules in a relatively slender vessel.

The deep jugular (*j.'*, fig. 11) arises in the Y-shaped space separating the orbital (*oa.*) from the internal carotid arteries (*i.c.*). It extends posteriorly parallel to the internal carotid artery and under the first efferent and along the median side of the otic capsule. A branch of it may join the superficial jugular just behind the ear capsule, but the union of the two may be made farther back, halfway between the entrance of the second and third efferents to the dorsal aorta. This vessel carries a good many corpuscles, but its source is so deep that I have had difficulty in ascertaining just where these come from.

THE SUPERIOR JUGULAR VEIN (ANTERIOR CARDINAL)

Internal jugular vein—Monro, 1785.
Vena jugularis superior—Müller, 1839.
Vena vertebralis anterior s. jugularis—Stannius, 1846, 1854.
Veine jugulaire—Milne-Edwards, 1858.
Veine jugulaire commune—Julin, 1887.
Vena jugularis–Owen, 1866; Huxley, 1871.

THE SUPERIOR JUGULAR (*sj.*, fig. 9) or anterior cardinal vein is formed by the union of the superficial and the deep jugular veins, after the former has received the posterior cerebral vein and the hyoidean sinus. On its course to the heart, the superior jugular, like the inferior jugular, receives numerous segmental veins (*sg.*, fig. 9).

Near their termini, right and left superior jugulars broaden out as sinuses near the midline and drop down through the ara of pronephric funnels into the sinus venosus (*ac.*, fig. 3). Thus, much of the blood which we have followed forward from the heart is returned to the heart through these great channels.

SUMMARY

THE STUDY OF THE CIRCULATION in a transparent type like Ammocoetes, in which one can observe individual corpuscles thread their way through even their terminal vessels, offers marked advantage over a study by dissection, where only the grosser vessels appear.

The changes in the form of the heart from the 2½ to 6½ mm. stages were studied, and the activity of the heart was followed as it carried forward the first bit of colorless plasma and on to the stage in which the force of its increased beat drove a great mass of corpuscles to all parts of the body.

The dying heart was observed and the endurance of the auricle especially noted.

The vessels and the circulating blood in the gills were studied in detail in larvae of from 7 to 10 mm. in length.

The external carotid artery on each side is formed from two vessels which are the ventral prolongations of the lower ends of the second and third efferent arteries. Oxygenated blood, which can be seen to pass through two or three of the ventral gills of the second and third arches, supplies this vessel.

The circulation in the endostyle was followed and the course of the blood stream charted.

The divisions of the orbital artery, formerly designated as the external carotid, and the passage of blood through these divisions, has been observed in numerous specimens, and the relation of these terminal branches on the side of the face to the external carotid artery is figured accurately. Moreover, the changes which take place between the 9 and 10 mm. stages in the terminal relations of the vein common to these two systems is described.

A discussion of the evolution of the orbital (stapedial) artery from the cyclostomes to man is added.

The most spectacular feature in the circulation of Ammocoetes is the course of blood over the brain. Corpuscles may be observed to enter from the terminal arteries under or at the sides of the brain, into the great sinuses, and to pass in parallel currents back over the divisions of the brain and pour down through great funnels on each side behind the ear capsules into the superior jugular veins.

All this blood may return to the heart dorsally by way of the superior jugular system, or some of it may pass down the hyoid sinus and into the heart through the inferior jugular system.

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CILIATES FROM BOS GAURUS H. SMITH

BY

C. A. KOFOID AND JOHN F. CHRISTENSON

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INTRODUCTION

THIS PAPER deals with the ciliate fauna of the stomach of a relict member of the ox tribe, *Bos gaurus*. This animal is related to the wisent, *Bison bonasus* Linnaeus, of the Russian forests. The gaur still exists in southern Asia on slopes of the Himalayan Mountains, in the Nilgiris, and in the Malay States. A few individuals were still to be found in Java in 1916.

The gaur represents a persisting wild type of the ox tribe, never domesticated, and still resistant to domestication. A few specimens are known in zoölogical gardens, and these have been captured while still young and reared under conditions of restraint. We were told at Mysore that the single specimen in the Zoölogical Gardens of that city, captured while young and reared in the Gardens, still retained its feral characteristics.

Since this wild ox has never existed under conditions of domestication, and since its range in pasturage is confined to primeval forests where domesticated cattle rarely enter, it might be expected that the ciliate fauna of its stomach would represent a more primitive, or at least a more restricted, type of infection than that found in domesticated cattle. It was hoped that the ciliate fauna of its stomach might throw some light upon the nature of primitive fauna of the ox tribe and upon the effects of the isolation of this species from the domesticated types of oxen.

The fauna of the stomach of the gaur does not, as we had hoped, show any notable distinctions from that of domesticated cattle. The same genera occur in it as in domesticated cattle, and eleven species found in its stomach are identical with those occurring in the stomach of Indian cattle. These eleven species and nine others not found in Indian cattle occur also rather widely among domesticated cattle elsewhere in the world. There are five new species found in the gaur, but they are closely related to species known in Indian cattle and other domesticated oxen. There is no evidence in the composition of the ophryoscolecid fauna in the gaur of any distinctive or notably characteristic line of evolution among these ciliates.

The existence of this similarity of the ciliate fauna in this isolated member of the ox tribe to that of other oxen suggests either (1) that the components of the fauna had been evolved and differentiated among primitive oxen prior to their separation in later evolutionary differentiation, or (2) that the line of evolution followed has been similar in the different host species after their evolutionary separation, or (3) that, in the face of the isolation resulting from the separation of feral and domesticated oxen, there has still been contact sufficient to provide for cross-infection and for commingling of the ciliate faunas of the stomach of the two groups. Of these three possibilities, the first and second can never be tested; the third has at least the support of the fact that faunas of ruminants are to at least a considerable degree transferable under experimental conditions.

HOST AND METHODS OF COLLECTING

THE HOST was *Bos gaurus* H. Smith, an old bull from the sandalwood and teak forests of H.R.H., the Maharajah of Mysore. The bull was an aged outcast from the small remaining herd in the Mysore jungle near the Nilgiri Mountains in Mysore State, India. This herd was reputed at the time to be composed of about eighty members, some twenty of which were seen by us in our visit to the jungle. They had been protected, and no shooting of them within the forest reserves of Mysore State had been permitted for years. The bull was taken by permission of the Maharajah and under the supervision of Mr. Rao, Game Conservator of Mysore State.

The herd was still exceedingly wild and difficult of approach. The bull was tracked by the two official game watchers of the forest, native men skillful in finding animals in the jungle. The trail was picked up about five o'clock in the morning at a point where the bull had crossed the jungle path, grazing on the very scanty, dew-laden grass. He was detected grazing in the scrub. Upon the first shot the bull at once snorted, lowered his massive head, and charged with great rapidity down upon the advance members of our party.

Mr. F. W. F. Fletcher, in charge of our party, shot the animal, but it took three bullets from a high-velocity cordite rifle of 0.450 calibre to bring him finally to earth.

The animal was a magnificent specimen of the gaur, with the typical almost hairless skin, roan color, and four symmetrical white "stockings." The height, measured on the carcass, was 6 feet, 6 inches, at the shoulder, and the weight was estimated at 2200 pounds.

The body was opened up immediately and the fluid from the four compartments of the stomach drained separately, fixed in hot Schaudinn's fluid while still warm, and later transferred to 70 per cent alcohol browned by the addition of Lugol's solution.

REVIEW OF THE LITERATURE

THE FAMILY OPHRYOSCOLECIDAE according to Dogiel (1927) consisted of 7 genera (*Entodinium, Diplodinium, Ophryoscolex, Opisthotrichum, Epidinium, Cunhaia,* and *Caloscolex*) and 117 species. Kofoid and MacLennan (1932) restricted the genus *Diplodinium* and revised it into 10 genera on the basis of significant differences in skeletal pattern, and (1933) erected a new genus *Epiplastron* to include species similar to *Epidinium* in form but with a skeletal complex of five parallel plates on the right and ventral surfaces. As conceived by Kofoid and MacLennan (1930, 1932, 1933), the ophryoscolecid fauna from *Bos indicus* consists of 14 genera, as follows: *Entodinium* Stein, *Diplodinium* Schuberg sens. str., *Eodinium* Kofoid and MacLennan, *Eremoplastron* K. and M., *Eudiplodinium* Dogiel, *Diploplastron* K. and M., *Metadinium* Awerinzew and Mutafowa, *Polyplastron* Dogiel, *Elytroplastron* K. and M., *Ostracodinium* Dogiel, *Enoploplastron* K. and M., *Epidinium* Crawley, *Epiplastron* K. and M., and *Ophryoscolex* Stein. Three other genera, *Caloscolex* Dogiel, *Opisthotrichum* Buisson, and *Cunhaia* Hasselmann, added to the 14 given above, make up a total of 17 valid genera of ciliates in the family. In addition to the 118 species recorded by Dogiel (1927) 45 new ones have since been described, 28 by Kofoid and MacLennan (1930, 1932, 1933), 6 by Dogiel (1928, 1932), 8 by Wertheim (1932, 1933), and 3 by Hsiung (1931, 1932). With the 5 described in the present paper there are now 168 species in the family Ophryoscolecidae. Of this number 25 were found in the stomach of the gaur as compared to 62 species recorded by Kofoid and MacLennan (1930, 1932, 1933) from *Bos indicus*. This larger number is in part a function of the larger number of stomachs examined in the latter species.

CHARACTERISTICS OF THE OPHRYOSCOLECIDAE FROM BOS GAURUS

Eleven species of ciliates found in *Bos gaurus* were reported also from *B. indicus* by Kofoid and MacLennan (1930, 1932, 1933). A comparison of these species common to the two hosts shows certain significant differences in 10 of the 11 species.

Differences

Species	From *Bos gaurus*	From *Bos indicus*
Entodinium nanellum	Body relatively stouter	Body relatively longer
Entodinium acutonucleatum	Macronucleus variable Spines relatively longer Notch between ventral spines deep	Macronucleus not very variable Spines relatively short Notch between ventral spines shallow
Entodinium longinucleatum	Macronucleus uniformly long	Macronucleus variable in length
Entodinium indicum	No significant differences	No significant differences
Epidinium caudatum	Body absolutely smaller	Body absolutely larger
Epidinium quadricaudatum	Body absolutely smaller Body relatively stouter	Body absolutely larger Body relatively longer
Eremoplastron rostratum	Body absolutely smaller Body relatively stouter Spine relatively shorter	Body absolutely larger Body relatively longer Spine relatively longer
Eudiplodinium maggii	Body absolutely smaller Body relatively stouter Mouth relatively wider	Body absolutely larger Body relatively longer Mouth relatively narrow
Metadinium medium	Body absolutely smaller Mouth relatively wider	Body absolutely larger Mouth relatively narrow
Ostracodinium gracile	Body absolutely smaller Body relatively stouter	Body absolutely larger Body relatively longer
Ostracodinium trivesiculatum	Body absolutely smaller Body relatively stouter	Body absolutely larger Body relatively longer

There is a very pronounced tendency for specimens from the gaur to be characteristically smaller and proportionately shorter and stouter than the specimens of the same species from *B. indicus*. This difference is most significant in the larger, more highly organized species such as *Epidinium quadricaudatum, Eudiplodinium maggii, Ostracodinium gracile,* and *Ostracodi-*

nium trivesiculatum. In ***Epidinium caudatum*** and ***Metadinium medium*** the differences are only in absolute size. Comparable size differences were not found by Kofoid and MacLennan between different hosts within the species ***B. indicus.***

A second tendency in which species from the gaur differ from the same species from the Indian ox relates to caudal spination. Thus, ***Entodinium acutonucleatum*** from ***Bos gaurus*** possesses spines which are both absolutely and relatively longer than those of the same species from ***B. indicus.*** This condition is reversed, however, in the single spine of ***Eremoplastron rostratum,*** since the spine of specimens from the Indian ox is longer, absolutely and relatively, than that of specimens from the gaur. Again, the spine of ***Epidinium caudatum*** of specimens from both hosts corresponds closely in length. It is clear that no generalization can be made respecting quantitative differences in the spination of specimens from the two hosts.

In ***Eudiplodinium maggii*** and ***Metadinium medium*** the mouth is relatively wider in specimens from the gaur than in those from the Indian ox. It is not known to what extent the contraction resulting from fixation may influence the size of the mouth, though the correspondence in sizes of the mouth for other species and in the common mode of fixation of specimens from both hosts indicates that these are definite quantitative differences.

The table on page 344 shows the differences between the specimens of species occurring in both *Bos gaurus* and *B. indicus.*

The following table summarizes the differences between ***Diplodinium monacanthum*** from ***Bos gaurus*** and the species reported as ***D. monacanthum*** Dogiel 1927 from ***B. indicus*** (K. and M., 1932). These differences, since they include a qualitative, seemingly heritable difference, the cuticular fold, are sufficient to justify the designation of the species from *B. indicus* as new. The species described from *B. indicus* as *D. monacanthum* Dogiel 1927 we now designate as ***D. ceylonicum*** sp. nov.:

DIFFERENCES

Diplodinium monacanthum from ***B. gaurus***	***Diplodinium ceylonicum*** sp. nov. from ***B. indicus***
Cuticular fold present	Cuticular fold absent
Body absolutely smaller	Body absolutely larger
Body relatively stouter	Body relatively longer
Spine absolutely longer	Spine absolutely much shorter
Spine relatively longer	Spine relatively very short

In addition to the 11 species which occur in both ***B. gaurus*** and ***B. indicus*** 5 new species are described from ***B. gaurus: Entodinium contractum, E. curtum, Metadinium rotundatum, Ostracodinium gauri,*** and ***O. mysorei.*** Also, 9 species were found in ***B. gaurus*** which were not found in ***B. indicus,*** and these are redescribed: ***D. monacanthum, D. diacanthum, D. triacanthum, D. tetracanthum, D. pentacanthum, D. anisacanthum, D. minor, Eodinium bilobosum,*** and ***Epidinium parvicaudatum.***

OCCURRENCE OF GENERA AND SPECIES IN BOS GAURUS

THE FOLLOWING TABLE shows the relative abundance of the species of Ophryoscolecidae found in *Bos gaurus*. In the first count (I) 100 specimens of the genus *Entodinium* are recorded while all the other Ophryoscolecidae and Holotricha are lumped indiscriminately. The second count (II) includes only 100 specimens of the higher Ophryoscolecidae. This method offers an effective means of showing the numerical preponderance of specimens of the genus *Entodinium*, as well as an opportunity to compare these figures with those taken from specimens from *B. indicus* by Kofoid and MacLennan (1930, 1932, 1933):

LIST OF OPHRYOSCOLECIDAE FROM THE STOMACH OF *Bos gaurus*

Species	From 2d stomach	From 3d and 4th stomachs
I. *Entodinium*		
Entodinium acutonucleatum	31	18
contractum	5	7
curtum	7	1
indicum	3	4
longinucleatum	2	4
nanellum	52	66
Total for *Entodinium*	100	100
Other Ophryoscolecidae	54	68
Holotricha	40	13
II. Higher Ophryoscolecidae		
Diplodinium minor	15	9
monacanthum	30	20
diacanthum	1	Pr.*
triacanthum	....	Pr.
tetracanthum	....	Pr.
pentacanthum	....	Pr.
anisacanthum	....	Pr.
Eodinium bilobosum	Pr.	1
Epidinium caudatum	10	7
quadricaudatum	....	Pr.
parvicaudatum	1	Pr.
Eremoplastron rostratum	2	2
Eudiplodinium maggii	12	11
Metadinium medium	4	3
rotundatum	2	6
Ostracodinium gauri	3	8
gracile	12	16
mysorei	3	10
trivesiculatum	5	7
Total	100	100

* Pr. indicates that the species is present but was not found during the count.
Compare I with Kofoid and MacLennan (1930).
Compare II with Kofoid and MacLennan (1932, 1933).

Entodinium acutonucleatum Kofoid and MacLennan 1930

Plate 25, figure 1; figure A, 1–2

Diagnosis.—See Kofoid and MacLennan (1930, p. 503).

Variations.—Specimens of this species taken from *B. gaurus* show no important variations in shape and proportions of spines, the same constancy having been noted for examples from *B. indicus* by Kofoid and MacLennan (1930). Greater variation, however, occurs in the macronucleus of specimens from *B. gaurus* than in those from *B. indicus*, since in the former host the

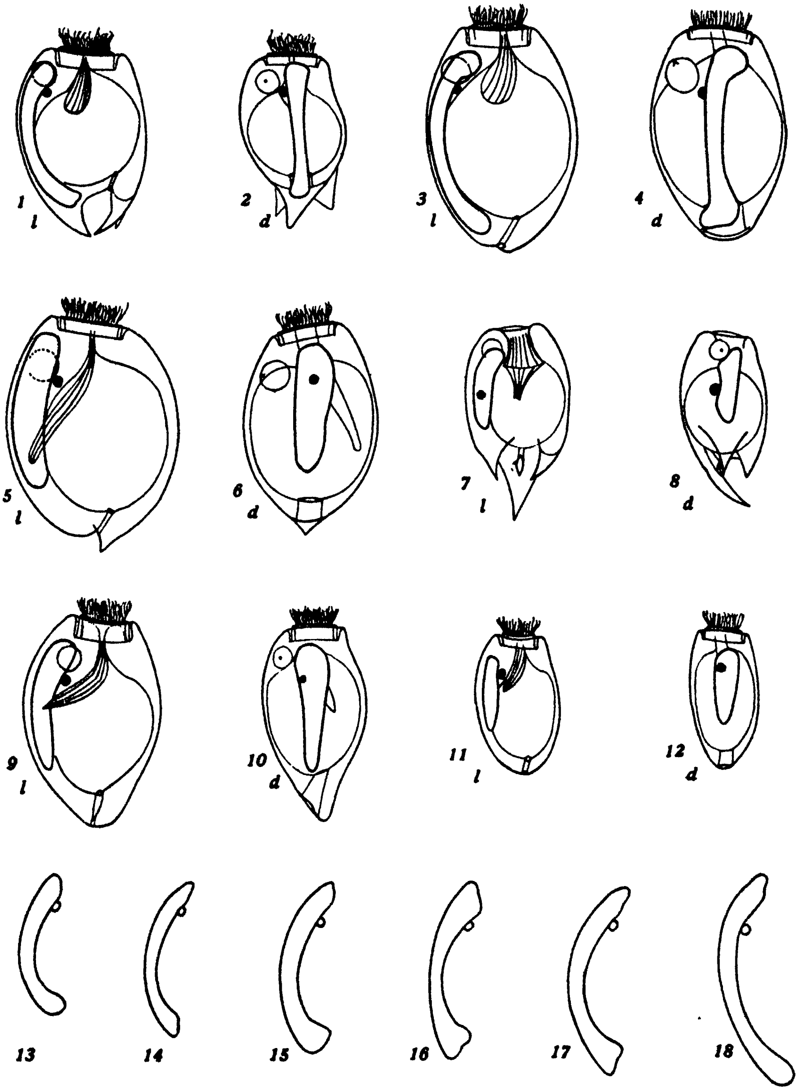

Fig. A. 1 and 2. *Entodinium acutonucleatum* Kofoid and MacLennan 1930; 3 and 4, *E. longinucleatum* Dogiel 1925; 5 and 6, *E. curtum* sp. nov.; 7 and 8, *E. indicum* Kofoid and MacLennan 1930; 9 and 10, *E. contractum* sp. nov.; 11 and 12, *E. nanellum* Dogiel 1922; 13–18, macronucleus of *E. acutonucleatum* showing variation in size and shape. *l*, right lateral view; *d*, dorsal view. × 600.

specimens have macronuclei with all intergradations from perfectly rounded ends to those with very slightly bilobed ends (fig. A, 13–18).

Comparison with same species from B. indicus.—There are two striking differences between individuals of *Entodinium acutonucleatum* from *B. gaurus* and those from *B. indicus*. First, the macronucleus of specimens from the former host shows greater variation in shape at the extremities, as was pointed out under Variations. Second, the mean length of the subequal spines, measured from the anal opening to the tip of the longest spine, is greater, 8(7–10)μ or 0.33(0.27–0.48) of the dorso-ventral diameter in length, in specimens from the gaur, than in specimens from the Indian ox, which possess spines 5(3–6)μ long or 0.19(0.13–0.27) of the dorso-ventral diameter in length. Coincident with this difference is the fact that the depth of the notch between the two ventral spines is also greater in specimens from *B. gaurus*, a fact evident only in a dorsal view of the animals.

Measurements.—Based on 10 individuals from *B. gaurus:* length, 30(26–36)μ; transdiameter, 21(19–24)μ; dorso-ventral diameter, 24(21–27)μ; length of macronucleus, 29(26–34)μ; dorso-ventral diameter of mouth, 14(12–16)μ; and length of tail, 8(7–10)μ.

Occurrence.—*E. acutonucleatum* has been reported by Kofoid and MacLennan (1930) from *B. indicus* from Coonoor, India, and Colombo, Ceylon, and it occurs in moderate numbers in *B. gaurus*.

Entodinium contractum sp. nov.

Plate 25, figure 4; figure A, 9–10

Diagnosis.—Only a single zone of membranelles, the adoral; body pyriform in lateral outline and elongated (1.39–1.66 dorso-ventral diameters in length); dorsal and ventral surfaces smoothly convex in anterior two-thirds of body, leveling out gradually in posterior third; posterior end of body smoothly rounded; macronucleus a curved, club-shaped rod, broader and deeper in anterior half; oesophagus a curved bundle, extending posteriorly to the right and dorsally; anus a characteristic funnel-shaped opening to the left of the posterior extremity. Length of 10 specimens from *B. gaurus*, 43(39–46)μ.

Description.—*E. contractum* possesses a characteristic pear-shaped body, 1.48(1.39–1.66) dorso-ventral diameters in length and compressed laterally to 0.83(0.75–1.00) of the dorso-ventral diameter. It presents the appearance of having a contracted posterior end. Both dorsal and ventral surfaces are smoothly convex in the anterior two-thirds of the body. In the posterior third, however, both surfaces lose their convexity and assume a linear course toward the posterior end, which is smoothly rounded. This characteristic shape is also evident in a dorsal view of the animal, though the right side is slightly more convex than the left.

A broad oral region occupies the anterior face of *E. contractum* and measures 0.48(0.41–0.55) of the dorso-ventral diameter. The mouth is tilted about 4° both ventrally and to the left of the main axis of the body.

The macronucleus is a characteristic, club-shaped rod, 1.07(0.90–1.24) dorso-ventral diameters in length, lying directly along the dorsal mid-line. It extends along the dorsal curvature of the body from the base of the outer adoral lip posteriorly to the beginning of the posterior fourth of the body. Both ends are smoothly rounded, but the anterior half is about twice as deep and twice as broad as the posterior half. The micronucleus is a small, ovoid to subspherical body, lying directly beneath and on the left margin of the macronucleus, one-fourth of the distance from its anterior end. Observed from lateral view the micronucleus lies within the acute angle formed by the oesophagus and the macronucleus.

A single contractile vacuole lies to the left of the anterior end of the macronucleus against the dorsal surface. It opens dorso-laterally through a small excretory pore.

The oesophagus is represented by an elongated bundle of fibrils, extending from the oral region posteriorly and dorsally to the right. This structure ascends steeply at an angle of about 40° and terminates sharply to the right of the center of the macronucleus. A deeply staining fibril, the ventral motor strand, can be traced around the oesophagus just beneath and inside of the outer adoral furrow.

A spacious endoplasmic sac is enclosed by a well defined boundary layer, which clings closely to the outer wall laterally and ventrally. On the dorsal surface the boundary layer forms a groove underlying the macronucleus and vacuole, and posteriorly it leaves the outer wall to continue to the point of origin of the rectum. Consequently, the posterior end contains a deep layer of ectoplasm, in contrast to the shallow layer elsewhere in the body. Anteriorly, the boundary layer merges with the wall of the oesophagus near its point of origin.

The rectum is a very short, dorso-ventrally depressed tube, and it is marked by a heavy rectal sphincter fibril at its point of origin from the endoplasm. A relatively large, conspicuous anus is one of the most important diagnostic features of this species. It consists of a prominent funnel-like opening entirely to the left of the posterior extremity of the body, so that it is invisible from a right lateral view.

Food.—Consists of bacteria and small flagellates.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 43(39–46)μ; transdiameter, 24(21–29)μ; dorso-ventral diameter, 29(27–34)μ; length of macronucleus, 31(27–36)μ; dorso-ventral diameter of mouth, 14(12–16)μ.

Occurrence.—Found in limited numbers in *B. gaurus.*

Relationships.—*E. contractum* is related to *E. bimastus* Dogiel 1927 in general body form, shape of macronucleus, and type of oesophagus. In *E. contractum,* however, the posterior end of the body is regularly tapered, and never drawn in abruptly to form a broad, rectangular lobe as in *E. bimastus.* In addition, the anus of *E. contractum* is always on the left surface of the posterior end, and never on the extremity as sometimes occurs in the related species.

Entodinium curtum sp. nov.

Plate 25, figure 5; figure A, 5–6

Diagnosis.—Body rotund and stout (1.21–1.56 dorso-ventral diameters in length); dorsal and ventral surfaces convex, with the ventral convexity most prominent; one short ventral spine on posterior end; macronucleus a slightly curved, stout, rod-like structure, extending about four-fifths of the length of the body; oesophagus long, curving upwards and to the right of the macronucleus. Length of 10 specimens from *B. gaurus,* 45 (40–53) μ.

Description.—The body of *E. curtum* possesses a marked rotundity, especially in the posterior part, and measures 1.37 (1.21–1.56) dorso-ventral diameters in length, and 0.85 (0.72–0.97) of the dorso-ventral diameter in transdiameter. Both ventral and dorsal sides of the body are symmetrically convex, the ventral one showing it to the greater degree. Anteriorly, the dorsal convexity becomes even less prominent, so that at the point of union with the oral face a sharp angle is formed. On the posterior end the ventral border merges into a small spine, 0.09 (0.06–0.12) of the dorso-ventral diameter in length, which is just below and slightly beyond the level of the anus. Dorsally it forms a small shelf whose base is 2.8 times its length and whose dorsal face has a shallow concavity which slopes toward the anal opening.

The mouth of *E. curtum* is tilted to the left at an angle of about 10°, and deviates ventrally about 6° from the longitudinal axis of the body. The oral area is circular and occupies a large part of the anterior face of the animal. It is 0.37 (0.28–0.44) of the dorso-ventral diameter in diameter.

The macronucleus is an elongate, rod-like body, 0.97 (0.73–1.21) of the dorso-ventral diameter in length, lying directly along the dorsal mid-line. It has a constant shape and is fairly constant in size. Occasionally the macronucleus extends anteriorly almost to the end of the body, but typically it ends near the bottom of the outer adoral furrow. It is rounded at both ends, follows the dorsal contour of the body, and ends posteriorly about 0.8 of the distance to the posterior end. Its depth remains nearly the same throughout its length, in contrast to the slight anterior lateral thickening evident from a dorsal view, from which the macronucleus appears wedge-shaped. The small, subspherical micronucleus lies directly beneath and in closs proximity to the ventral surface of the macronucleus, 0.25 of its length posterior to its anterior end.

The single contractile vacuole is in the ectoplasmic layer to the left of the anterior end of the macronucleus, its posterior end reaching to, or slightly beyond, the level of the micronucleus. It opens to the exterior through a minute excretory pore, which is to the left of the dorsal mid-line.

The boundary layer, enclosing the endoplasm, lies directly underneath the pellicle on the lateral and ventral surfaces of the body. Its even contour is interrupted dorsally, where it forms a concavity encasing the ventral face of the macronucleus and the contractile vacuole.

A long, curved oesophagus is present in *E. curtum.* From its point of origin at the center of the oral region it progresses posteriorly, curves to the right and dorsally at an angle of about 30°–40°, and ends on a level with the beginning of the posterior third of the macronucleus. This posterior termination is far over against the right side of the body. The wall of the oesophagus is composed of a number of feebly staining longitudinal fibrils, and forms a characteristic feature of this species.

The anal opening lies on the extreme posterior end of the animal, just dorsal to the small spine, and is a relatively wide, dorso-ventrally depressed slit. It opens to the exterior from a sloping rectum, which tilts upward at an angle of 25° in making its passage from the endoplasm. At the point of junction of the rectum with the endoplasmic sac there occurs a heavy fibril, surrounding the opening into the rectum.

Food.—Bacteria and very small pieces of plant debris comprise its food.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 45(40–53)μ; transdiameter, 28(24–33)μ; dorso-ventral diameter, 33(26–38)μ; length of macronucleus, 32(27–38)μ; dorso-ventral diameter of mouth, 12(10–15)μ; length of spine, 3(2–4)μ.

Occurrence.—Found in small numbers in *B. gaurus.*

Relationships.—Is most closely related to *E. ellipsoideum.* These two species agree closely in general body form and proportions, in shape of macronucleus, and type of oesophagus. The significantly smaller size and the short, diagnostic, ventral spine of *E. curtum,* however, differentiate it sharply from *E. ellipsoideum.*

Entodinium indicum Kofoid and MacLennan 1930

Plate 25, figure 2; figure A, 7–8

Diagnosis.—See Kofoid and MacLennan (1930, p. 533).

Variations.—This species is characterized by constancy in body shape, spination, and size and shape of the macronucleus.

Comparison with same species from B. indicus.—No significant differences were found to exist in this species in these two hosts.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 33(28–37)μ; transdiameter, 20(17–22)μ; dorso-ventral diameter, 23(20–26)μ; length of macronucleus, 23(17–32)μ; length of large spine, 12(10–16)μ.

Occurrence.—Found by Kofoid and MacLennan (1930) in *B. indicus* from Coonoor, India, and Colombo, Ceylon, and in limited numbers in *B. gaurus.*

Entodinium longinucleatum Dogiel 1925

Plate 25, figure 3; figure A, 3–4

Diagnosis.—See Kofoid and MacLennan (1930, p. 501).

Comparison with same species from B. indicus.—The constancy in specimens from *B. gaurus* is in sharp contrast to the condition in individuals reported from *B. indicus by* Kofoid and MacLennan (1930), which showed a striking degree of variability in the morphology of the macronucleus, all intergradations between those with short nuclei to those with the typical *E. longinucleatum* nucleus being found. In all specimens examined from *B. gaurus* the macronucleus was found to be constantly very long, 1.28(1.16–1.43) dorso-ventral diameters in length, extending from the anterior end of the body nearly to the anal slit. In all these specimens from the gaur both anterior and posterior ends of the macronucleus are twice as broad as the middle part, and the anterior end is slightly deeper.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 43(35–50)μ; transdiameter, 24(21–29)μ; dorso-ventral diameter, 29(25–32)μ; length of macronucleus, 37(29–42)μ; dorso-ventral diameter of mouth, 14(13–17)μ.

Occurrence.—In addition to the localities given by Kofoid and MacLennan (1930) *E. longinucleatum* has been found in sheep from China by Hsiung (1931) and in limited numbers in *B. gaurus.*

Entodinium nanellum Dogiel 1922

Plate 25, figure 6; figure A, 11–12

Diagnosis.—See Kofoid and MacLennan (1930, p. 524).

Variations.—There are no significant variations in specimens from *B. gaurus,* as was also noted for those from *B. indicus* by Kofoid and MacLennan (1930).

Comparison with same species from B. indicus.—Measurements show that the individuals of this species taken from *B. gaurus* are relatively stouter than those taken from *B. indicus.* Specimens from the former host are 1.61(1.44–1.75) dorso-ventral diameters in length, while those from the latter are 1.76 (1.50–2.00) dorso-ventral diameters in length. It is interesting to note that Dogiel (1927) has reported individuals of this species from *Aepyceros melampus* from British East Africa having a value of 2.00 for this same relationship. In morphological characteristics there are no noticeable differences between individuals from *B. gaurus* and those from *B. indicus.*

Measurements.—Based on 10 specimens from *B. gaurus:* length, 29(25–35)μ; transdiameter, 14(12–17)μ; dorso-ventral diameter, 18(16–20)μ; length of macronucleus, 17(13–23)μ; dorso-ventral diameter of mouth, 9(7–10)μ.

Occurrence.—Occurs abundantly in *B. gaurus.*

Diplodinium minor Dogiel 1925

Plate 25, figure 8; figure B, 3–4

Diagnosis.—Body oval, truncated anteriorly, relatively stout (1.22–1.51 dorso-ventral diameters in length), and somewhat triangular in lateral view in the posterior third; dorsal zone of membranelles lies at same transverse level of body as adoral zone; a narrow, longitudinal cuticular line on right dorso-lateral surface, extending from base of outer dorsal furrow to dorsal edge of anal opening; rectum a dorso-ventrally depressed canal opening to exterior through inconspicuous slit-like anus on posterior end; endoplasmic sac does not extend anteriorly into operculum. Length, 53–90μ; length of 10 specimens from *B. gaurus,* 60(53–80)μ.

Description.—The body of *D. minor* is relatively stout (1.22–1.51 dorso-ventral diameters in length) and strongly compressed laterally (0.68–0.81 of the dorso-ventral diameter in transdiameter). In lateral outline it appears oval, though truncated anteriorly and somewhat triangular in the posterior third. The greatest dorso-ventral diameter occurs in the mid-body region. The ventral surface of the body is more strongly convex than the dorsal surface, especially in the part of the body just posterior to the middle. The dorsal surface maintains a constant convexity from the dorsal membranelle zone to the roof of the anal aperture. From the dorsal view the lateral surfaces of the body appear almost equally convex, both tapering gradually toward the smooth posterior extremity. Anteriorly, these lateral convexities merge continuously with the rounded operculum. The greatest transdiameter of the body is found a short distance posterior to the level of the micronucleus, at about 0.4 of the body length behind the apex of the anterior end. A distinct, longitudinal, cuticular groove, appearing as a dark line, extends along the dorso-lateral part of the right surface from the dorsal zone to the dorsal wall of the anal aperture.

The oral region is of moderate size, 0.40(0.28–0.49) of the dorso-ventral diameter in diameter, and is tilted both ventrally and laterally to the left at angles of about 25° from the main axis. A shallow operculum projects anteriorly a short distance beyond the oral zone and separates it from the dorsal zone of locomotor membranelles. This dorsal zone lies at the same transverse level of the body as the adoral zone and is tilted to the left of the main axis at an angle of about 10°.

A typical *Diplodinium* macronucleus lies under the right surface of the body, slightly dorsal to the lateral mid-line. This structure is relatively stout, 0.71(0.65–0.93) of the dorso-ventral diameter in length, and the anterior third is bent ventrally at an angle of 45°, presenting a somewhat hatchet-like appearance. The dorso-ventral diameter in the anterior ventrally curved portion is twice as great as in the posterior third, while the transverse diameter is only slightly greater in the anterior third than in the posterior third. Therefore, from dorsal view the macronucleus appears somewhat cuneiform. The posterior surface is small and smoothly rounded, but the anterior surface, while typically smooth, shows intergradations toward an irregular surface (fig. B, 5–10). On the antero-dorsal surface the macronucleus is slightly concave. A small, ovoid micronucleus lies in this concavity near the left margin of the macronucleus.

There are two contractile vacuoles lying on the dorsal mid-line of the body. The forward vacuole is at the transverse level of the micronucleus, while the posterior one is at the beginning of the posterior third of the body, or nearly on the level of the posterior end of the macronucleus. Both vacuoles lie to the left of the macronucleus. Usually, the posterior vacuole is much smaller than the anterior one, and both usually present an ellipsoidal appearance resulting from dorso-ventral depression. Each possesses a short excretory duct leading to the exterior through a small pore on the mid-dorsal surface.

An inconspicuous oesophagus is weakly marked by several curved, longitudinal fibrils which extend from the mouth postero-dorsally at an angle of 15° and to the right at an angle of 25° from the main axis. These fibrils terminate on the right surface near the middle of the body and just ventral to the posterior third of the macronucleus.

A spacious endoplasmic sac is clearly delimited by a well defined boundary layer. The regularity of this layer is interrupted by two small dorsal concavities in which lie the two contractile vacuoles, and by a large concavity on the right side containing the macronucleus. Anteriorly, the boundary layer tapers sharply to merge with the anterior part of the oesophagus. It makes no diverticulum into the operculum such as occurs in a related species, *D. costatum* Dogiel 1925. In the posterior end the boundary layer retreats from near the body surface to give rise to the rectum.

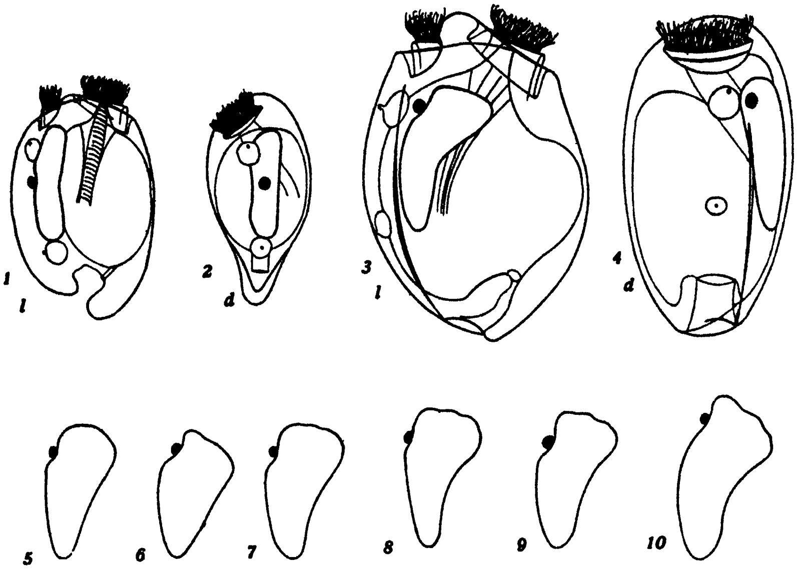

Fig. B. 1 and 2, *Eodinium bilobosum* (Dogiel 1927); 3 and 4, *Diplodinium minor* Dogiel 1925; 5–10, macronucleus of *D. minor* showing variability in size and shape. *l*, right lateral view; *d*, dorsal view. × 600.

The rectum is a poorly defined but strongly dorso-ventrally depressed canal, leading from the endoplasmic sac postero-dorsally at an angle of 30°. It flares out to form a broad, somewhat dorso-ventrally depressed anus, which lies on the extreme posterior end of the body in a conspicuous depression.

Food.—Many pieces of plant debris of variable sizes are found within its endoplasm.

Variations.—*D. minor* appears to vary significantly in only one feature, the macronucleus. The anterior surface of this structure varies from the typical form, which is smoothly rounded, to those in which this face assumes an irregular, somewhat lobate, contour (fig. B, 5–10). In addition, the ventral surface of the macronucleus shows intergradations from nearly straight to a considerably concave surface. Variation in size of body and size of macronucleus is considerable, but this is correlated with the stage in the life-cycle at which observations are made, since specimens approaching division are larger.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 60(53–80)μ; transdiameter, 33(30–42)μ; dorso-ventral diameter, 45(40–53)μ; length of macronucleus, 32(26–41)μ; dorso-ventral diameter of mouth, 18(15–21)μ.

Dogiel (1927) gives the following measurements for *D. minor:* length, 75 (60–90)μ; dorso-ventral diameter, 47(45–51)μ; relation of length to dorso-ventral diameter, 1.6.

Occurrence.—Occurs in the African antelope (*Rhaphiceros* sp.) and bushbuck (*Tragelaphus scriptus*) from British East Africa (Dogiel, 1925) and in moderate numbers in the gaur.

Relationships.—The presence of a distinct, longitudinal, cuticular line along the length of the right dorso-lateral surface of the body, and the relatively stout and oval appearance, relates *D. minor* to *D. rangiferi* Dogiel 1925, *D. dogieli* K. and M. 1932, and *D. costatum* Dogiel 1925. These four species comprise the natural *rangiferi* group (Kofoid and MacLennan, 1932).

Diplodinium monacanthum Dogiel 1927

Plate 26, figure 9; figure C, 1–2

Diagnosis.—See Kofoid and MacLennan (1932, p. 78). There is also a longitudinal cuticular groove along the right dorso-lateral surface.

Variations.—One individual with a bifurcated ventral spine was observed.

Comparison with the species described as Diplodinium monacanthum *from* B. indicus.—Specimens of *D. monacanthum* from *B. gaurus* differ from those described as *D. monacanthum* from *B. indicus* (K. and M., 1932) in body size and proportions, and in actual and relative length of the caudal spine. Individuals of this species from the gaur are 60(50–68)μ or 1.40(1.30–1.62) dorso-ventral diameters in length, 34(30–38)μ or 0.78(0.70–0.95) of the dorso-ventral diameter in transdiameter, and the caudal spine measures 13(10–17)μ or 0.30(0.23–0.40) of the dorso-ventral diameter in length. The specimens described as *D. monacanthum* from the Indian ox are 92(60–124)μ or 1.66(1.52–1.82) dorso-ventral diameters in length, 49(33–64)μ or 0.90(0.84–0.94) of the dorso-ventral diameter in transdiameter, and the caudal spine is very small, being only 6(5–8)μ or 0.10(0.08–0.13) of the dorso-ventral diameter in length. Differences in the sizes of macronuclei and mouth diameters are absolute only, and are concurrent with differences in body size of specimens from the two hosts.

There are other differences to be observed, one a qualitative difference, the presence of a cuticular groove in specimens from *B. gaurus. D. monacanthum* from the gaur was found to possess a distinct, longitudinal, cuticular groove on the right latero-dorsal surface, a structure not observed in the specimens described as *D. monacanthum* from the Indian ox. The relatively less tapered posterior end and the situation of the rectum farther dorsally from the postero-ventral border in the specimens from the gaur are correlated to the relatively shorter length of body and relatively larger ventral spine in these specimens.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 60(50–68)μ; transdiameter, 34(30–38)μ; dorso-ventral diameter, 43(39–45)μ; length of macronucleus, 32(24–39)μ; dorso-ventral diameter of mouth, 17(14–19)μ; length of spine, 13(10–17)μ.

Dogiel's (1927) measurements of this species from domestic cattle in the U.S.S.R. are intermediate between ours from the gaur and the Indian ox. Since there are no structural differences which distinguish the individuals from the gaur from those described by Dogiel we regard the difference in size as not systematically significant.

Occurrence.—Found in cattle from the U.S.S.R. by Dogiel (1927) and abundantly in *B. gaurus.*

Relationships.—This species is the second member of the *D. anacanthum* group. This species as described by Kofoid and MacLennan (1932) from the Indian ox lacks the cuticular groove, is distinctly and consistently larger, and has a shorter spine. It is now regarded as a distinct species and is here designated as *D. ceylonicum* sp. nov.

Diplodinium diacanthum Dogiel 1927

Plate 26, figure 10; figure C, 3–4

Diagnosis.—Morphologically similar to *D. monacanthum* in all respects except spination; two caudal spines present—a ventral one of the same size and position as the spine of *D. monacanthum,* and a second, smaller spine (on the right latero-ventral portion of the posterior end in 60 per cent, and on the dorsal side in 40 per cent of the specimens found in *B. gaurus*). Length, 50–83μ; length of 10 specimens from *B. gaurus,* 59(50–70)μ.

Description.—The relatively short and stout body of *D. diacanthum* is 1.40 (1.26–1.49) dorso-ventral diameters in length and is laterally compressed to 0.83(0.77–0.89) of the dorso-ventral diameter. It appears somewhat truncated anteriorly when viewed from the lateral aspect. The greatest dorso-ventral diameter of the ovoid portion of the body occurs midway between the anterior and the posterior ends. Both dorsal and ventral surfaces of the body are convex, but the dorsal convexity is the greater. The dorsal surface maintains its characteristic convexity throughout its entire length. The ventral surface is most convex in its middle third, while the anterior and posterior thirds often show slight concavities. Both surfaces taper posteriorly to give the characteristic appearance of members of the *D. anacanthum* group. In dorsal view the greatest transdiameter is observed to lie slightly posterior to the level of the micronucleus. Anteriorly, the slightly convex lateral surfaces merge into the shallow, regular operculum, while on the posterior end they taper gradually toward the anus.

A distinct, longitudinal, cuticular groove extends along the right latero-dorsal margin of the body from the dorsal membranelle zone to the posterior end, as in *D. monacanthum.*

An oral region of moderate size, 0.43(0.37–0.47) of the dorso-ventral diameter in diameter, occupies the anterior ventro-lateral portion of the body. It deviates ventrally at an angle of about 25° and to the left at an angle of about

20° from the main body axis. On the right side the outer adoral lip is continuous with the shallow operculum, which projects only slightly anteriorly to the level of the mouth, and which separates adoral from dorsal membranelle zones. The dorsal row of locomotor membranelles is tilted slightly to the left of the main axis.

There are two short spines on the caudal extremity of *D. diacanthum,* a feature distinguishing it from other species in the *D. anacanthum* series. One spine is ventral in position, similar in size and shape to the corresponding spine in *D. monacanthum,* and is 0.31 (0.26–0.38) of the dorso-ventral diameter in length. The extremity of this spine is curved dorsally in the mid-line of the body. The second spine is only half as long as the ventral one, and is situated either latero-ventrally on the right surface or directly opposite the ventral spine on the dorsal surface.

The stout, hatchet-shaped macronucleus, 0.71 (0.64–0.85) of the dorso-ventral diameter in length, lies directly beneath the right surface of the middle part of the body, slightly dorsal to the lateral mid-line. Its forward end is curved ventrally at an angle of 45°, as in most species of the genus *Diplodinium.* It is morphologically similar to the macronucleus of *D. minor* with the exception that in this species the anterior surface is always smoothly rounded. A small, subspherical micronucleus is situated in a slight concavity on the left antero-dorsal surface of the macronucleus.

Two contractile vacuoles lie along the dorsal mid-line, one at each extremity of the middle third of the body. They are often dorso-ventrally depressed. Typically, the posterior vacuole is much smaller than the anterior one, and often it is indistinguishable, though the pore may persist. A short excretory canal leads to the dorsal surface from each vacuole, opening through a small pore.

A weakly defined oesophagus extends posteriorly and to the right from the oral region to terminate beneath the posterior end of the macronucleus.

The endoplasmic sac is spacious, extending from the mouth to the posterior end of the body, and is separated from the outer surfaces of the body by a narrow layer of ectoplasm. The well defined boundary layer marking the limits of the endoplasm forms two dorsal concavities which contain the contractile vacuoles, and forms a larger lateral concavity on the right surface in which the macronucleus lies. The ectoplasm is thickest in the operculum and in the regions ventral to the origin of the oesophagus and the rectum.

A somewhat poorly differentiated rectum arises from the posterior termination of the boundary layer and is a short, dorso-ventrally flattened tube extending postero-dorsally at an angle of 30°. It terminates in a slit anus, which lies just dorsal to the base of the ventral spine.

Food.—Ingests pieces of plant debris.

Variations.—This species shows no great variability in general morphological characteristics. The arrangement of spines, however, is subject to variation. While the larger ventral spine is constant in position, the smaller second spine may be situated in two locations. In 60 per cent of the specimens observed from *B. gaurus* it is found in close proximity to the ventral spine on the right surface, or latero-ventrally. In the other 40 per cent of individuals this second spine lies directly opposite the ventral one, or dorsally. One specimen, in which the second spine was dorsal, possessed a bifurcated ventral spine.

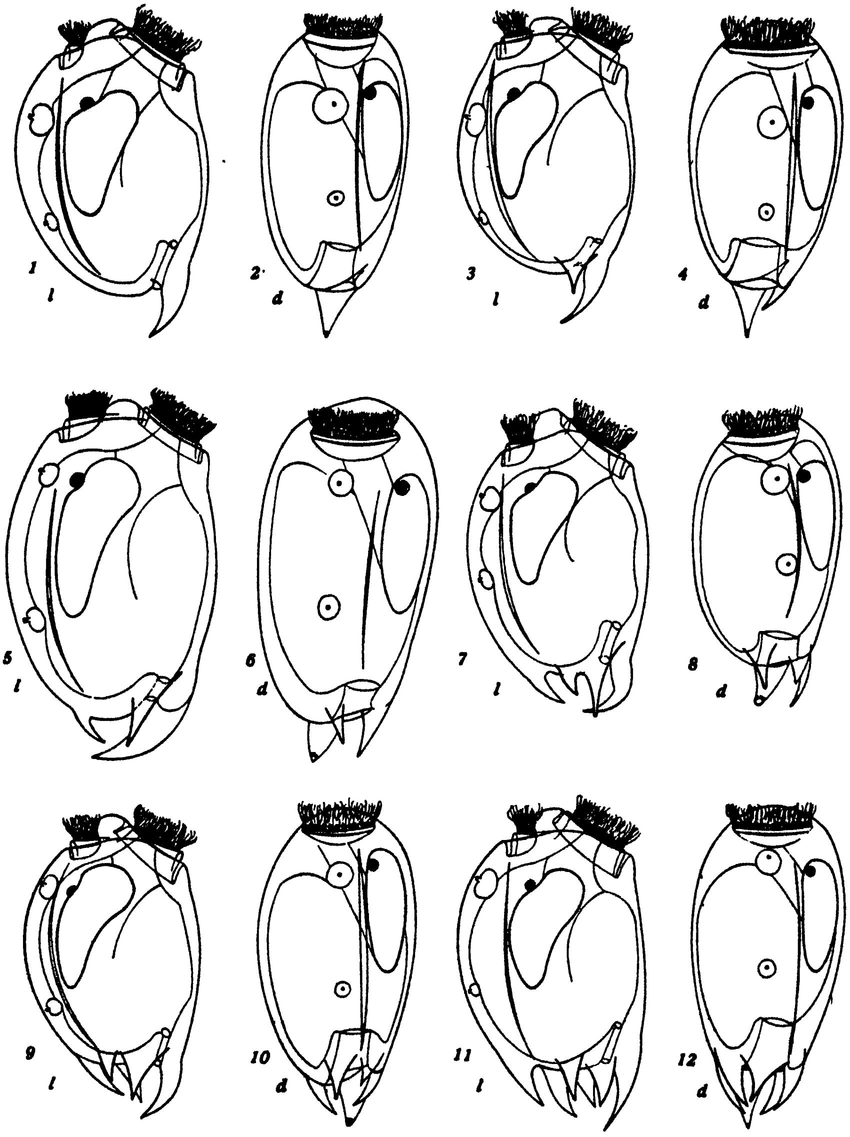

Fig. C. 1 and 2, *Diplodinium monacanthum* Dogiel 1927; 3 and 4, *D. diacanthum* Dogiel 1927; 5 and 6, *D. triacanthum* Dogiel 1927; 7 and 8, *D. tetracanthum* Dogiel 1927; 9 and 10, *D. pentacanthum* Dogiel 1927; 11 and 12, *D. anisacanthum* Cunha 1914. *l*, right lateral view; *d*, dorsal view. × 600.

In specimens of *D. diacanthum* from domestic cattle from the U.S.S.R. Dogiel (1927) observed that the second spine was dorsal, as in only 40 per cent of the specimens from *B. gaurus*. In only one specimen did Dogiel note another position for the second spine, this being latero-dorsal on the right side. This is sharply contrasted to the latero-ventral position on the right side of this second spine in 60 per cent of the specimens from *B. gaurus.*

Measurements.—Based on 10 specimens from *B. gaurus:* length, 59(50–70)μ; transdiameter, 35(33–42)μ; dorso-ventral diameter, 42(38–48)μ; length of macronucleus, 30(27–41)μ; dorso-ventral diameter of mouth, 18(15–21)μ; length of ventral spine, 13(10–16)μ.

Dogiel (1927) gives the following measurements for specimens taken from cattle from the U.S.S.R.: length, 75(70–83)μ; dorso-ventral diameter, 52(47–60)μ; relation of length to dorso-ventral diameter, 1.45.

Occurrence.—Found in domestic cattle from the U.S.S.R. by Dogiel (1927) and in small numbers in *B. gaurus.*

Relationships.—*D. diacanthum* is the third member of the *D. anacanthum* series, showing an additional caudal spine over the one-spined condition of *D. monacanthum.*

Diplodinium triacanthum Dogiel 1927

Plate 26, figure 11; figure C, 5–6

Diagnosis.—Morphologically similar to *D. diacanthum* in all respects except spination; three caudal spines present—one ventral, one dorsal, and one on the right latero-ventral side. Length, 64–85μ; length of specimen from *B. gaurus,* 64μ.

Description.—The morphological features of *D. triacanthum,* with the exception of spination, correspond closely to those given above for *D. diacanthum.*

This species shows an additional caudal spine over the two-spined condition in *D. diacanthum.* There is a relatively large ventral spine, 0.39 of the dorso-ventral diameter in length, corresponding to the single spine in *D. monacanthum.* A second, smaller spine is on the latero-ventral edge of the right side and corresponds to the second spine in 60 per cent of the specimens of *D. diacanthum* from *B. gaurus.* Finally, a third, small spine lies on the dorsal edge, opposite the ventral spine, and corresponds to the second spine in 40 per cent of the specimens of *D. diacanthum* from *B. gaurus.*

Variations.—Dogiel (1927) reported variability in the position of the spines in *D. triacanthum* taken from domestic cattle from the U.S.S.R. In 80 per cent of these specimens the spination corresponds exactly to that noted above in the individual from *B. gaurus.* The remaining 20 per cent, however, possessed ventral and dorsal spines as in the others, but the third spine was on the right latero-dorsal border of the posterior end.

Measurements.—Based on 1 specimen found in *B. gaurus:* length, 64μ; transdiameter, 38μ; dorso-ventral diameter, 46μ; length of macronucleus, 34μ; dorso-ventral diameter of mouth, 18μ; length of ventral spine, 18μ.

Dogiel (1927) gives the following measurements for *D. triacanthum* from domestic cattle from the U.S.S.R.: length, 77(70–85)μ; dorso-ventral diameter, 55(51–63)μ; relation of length to dorso-ventral diameter, 1.4.

Occurrence.—Found in domestic cattle from the U.S.S.R. by Dogiel (1927), but only a single specimen was found in *B. gaurus.*

Relationships.—*D. triacanthum* is the fourth member of the *D. anacanthum* series, showing an advance over the two-spined condition of *D. diacanthum* in the possession of three caudal spines.

Diplodinium tetracanthum Dogiel 1927

Plate 26, figure 12; figure C, 7–8

Diagnosis.—Morphologically similar to *D. diacanthum* in all respects except spination; four caudal spines present—one ventral, one dorsal, and two on the right side. Length, 53–83μ; length of 2 specimens from *B. gaurus,* 55 (53–56)μ.

Description.—Food habits and morphological features of *D. tetracanthum,* with the exception of spination, are similar to those given above for *D. diacanthum.*

D. tetracanthum is characterized by the possession of four spines on the posterior end. There is a large ventral spine, 0.35 of the dorso-ventral diameter in length, and similar to the single spine in *D. monacanthum.* A second spine is smaller and dorsal in position, as in 40 per cent of the specimens of *D. diacanthum* from *B. gaurus.* The third spine is on the latero-ventral part of the right side, as in 60 per cent of the specimens of *D. diacanthum* and in *D. triacanthum* from *B. gaurus.* The fourth spine is added on the latero-dorsal edge of the right side, distinguishing this species from the preceding four species of the *D. anacanthum* series.

Variations.—The typical spination in *D. tetracanthum* described above is subject to variation. Thus, in one specimen from *B. gaurus* there was no dorsal spine. Instead, a spine occurred on the latero-dorsal edge of the left side. The remaining three spines were in typical locations.

Dogiel (1927), in specimens taken from domestic cattle from the U.S.S.R., noted two exceptions to the usual arrangement of spines. In one the spines were ventral, dorsal, latero-ventral on the right side, and latero-ventral on the left side. In the other they were ventral, dorsal, latero-dorsal on the right side, and latero-dorsal on the left side.

Measurements.—Based on 2 specimens found in *B. gaurus:* length, 55(53–56)μ; transdiameter, 33(32–33)μ; dorso-ventral diameter, 40(38–41)μ; length of macronucleus, 28(26–29)μ; dorso-ventral diameter of mouth, 18(17–18)μ; length of ventral spine, 14(12–15)μ.

Dogiel (1927) gives the following measurements for specimens of this species taken from domestic cattle from the U.S.S.R.: length, 76(72–83)μ; dorso-ventral diameter, 54(52–61)μ; relation of length to dorso-ventral diameter, 1.4.

Occurrence.—Reported by Dogiel (1927) from domestic cattle from the U.S.S.R. Two specimens were found in *B. gaurus.*

Relationships.—*D. tetracanthum* is the fifth member of the *D. anacanthum* series, showing an advance over the three-spined condition in *D. triacanthum* by having four spines.

Diplodinium pentacanthum Dogiel 1927

Plate 26, figure 13; figure C, 9–10

Diagnosis.—Morphologically similar to *D. diacanthum* in all respects except spination; five caudal spines present—one ventral, one dorsal, two on the right side, and one on the left side. Length, 50–84μ; length of 2 specimens from *B. gaurus,* 52(50–54)μ.

Description.—*D. pentacanthum* differs from *D. diacanthum,* described above, in spination only.

There are five caudal spines in members of this species. The ventral spine is largest, 0.30 of the dorso-ventral diameter in length, and is similar to the single spine in *D. monacanthum.* The remaining spines are shorter. Of these, one is dorsal, two are on the right side, and the last occupies the median part of the left side. Thus, the spination in *D. pentacanthum* represents that in *D. tetracanthum* with an additional spine added on the left side.

Variations.—The arrangement of spines in the two specimens from *B. gaurus* showed no variation. Dogiel (1927), however, found that the spination varied in specimens from domestic cattle from the U.S.S.R. Typically, his examples possessed the arrangement of spines described above for the ciliates from the gaur, except that the fifth spine was on the latero-ventral part of the left side instead of median. Two exceptions to this usual arrangement were observed. In one, the single lateral spine was on the latero-ventral portion of the right side. In the other, the single lateral spine was latero-dorsal on the left side.

Measurements.—Based on 2 specimens found in *B. gaurus:* length, 52(50–54)μ; transdiameter, 33(32–34)μ; dorso-ventral diameter, 40(38–41)μ; length of macronucleus, 32(29–34)μ; dorso-ventral diameter of mouth, 15(14–16)μ; length of ventral spine, 12(11–13)μ.

Dogiel (1927) gives the following measurements for specimens found in domestic cattle from the U.S.S.R.: length, 77(67–84)μ; dorso-ventral diameter, 55(51–60)μ; relation of length to dorso-ventral diameter, 1.4.

Occurrence.—Found in domestic cattle from the U.S.S.R. by Dogiel (1927). Two specimens were found in *B. gaurus.*

Relationships.—*D. pentacanthum* is the sixth member of the *D. anacanthum* series, showing a progressive advance over the four-spined *D. tetracanthum* in the possession of five caudal spines.

Diplodinium anisacanthum Cunha 1914

Plate 26, figure 14; figure C, 11–12

Diagnosis.—Morphologically similar to *D. diacanthum* in all respects except spination; six caudal spines present—one ventral, one dorsal, and two on each side. Length, 46–86μ; length of 10 specimens from *B. gaurus,* 56(46–67)μ.

Description.—The morphological features already given for *D. diacanthum* correspond in all details except spination to those of *D. anisacanthum.*

There are six caudal spines in this species. The ventral spine is largest, 0.32

(0.29–0.36) of the dorso-ventral diameter in length, as in all other species of the *D. anacanthum* group having caudal armature. The remaining five spines are shorter and subequal in length. All spines exhibit incurving at their extremities. This species represents the spination of *D. pentacanthum* with a sixth spine added in the unoccupied space on the lateral surface. All available situations for caudal spines are occupied in *D. anisacanthum*, and no seven-spined forms have been observed in this genus.

Variations.—Individuals from *B. gaurus* were uniform, but Dogiel (1927) reported that in *D. anisacanthum* in some specimens from domestic cattle from the U.S.S.R. and Turkestan the two spines on the left surface had degenerated to mere cuticular humps.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 56(46–67)μ; transdiameter, 33(28–37)μ; dorso-ventral diameter, 41(38–47)μ; length of macronucleus, 31(25–40)μ; dorso-ventral diameter of mouth, 16(14–18)μ; length of ventral spine, 13(12–15)μ.

Dogiel (1927) reports the following measurements for *D. anisacanthum* from domestic cattle: length, 82(77–86)μ; dorso-ventral diameter, 52(53–61)μ; relation of length to dorso-ventral diameter, 1.5.

Occurrence.—Reported in domestic cattle from Brazil by Cunha (1914) and from the U.S.S.R. and Turkestan by Dogiel (1927). It occurs in very limited numbers in *B. gaurus.*

Relationships.—*D. anisacanthum* is the seventh and last member of the *D. anacanthum* series of species.

Eodinium bilobosum (Dogiel 1927)

Plate 25, figure 7; figure B, 1–2

Diagnosis.—Dorsal zone of small size, at same transverse level of body as adoral zone; body relatively stout (1.22–1.50 dorso-ventral diameters in length) and laterally compressed (0.77–0.92 of the dorso-ventral diameter in transdiameter); dorsal surface slightly convex, ventral surface somewhat flattened; two caudal lobes present—one dorsal and one ventral, lying in the same dorso-ventral plane; oesophagus a long, curved structure marked by conspicuous transverse membranelles, giving a ladder-like appearance to it. Length, 33–60μ; length of 10 specimens from *B. gaurus,* 39(33–43)μ.

Description.—The relatively short body of *E. bilobosum* (1.22–1.50 dorso-ventral diameters in length) is somewhat subrectangular in lateral view, and from the dorsal aspect the body is seen to be laterally compressed (0.77–0.92 of the dorso-ventral diameter in transdiameter). The oral area (0.37–0.52 of the dorso-ventral diameter in diameter) is tilted ventrally at an angle of 15°–20° and to the left at an angle of about 25° from the longitudinal axis of the body. A small, inconspicuous operculum separates the adoral membranelle zone from the dorsal zone, both of which lie on the same transverse level of the body. Since the dorsal zone lies parallel with the transdiameter of the mouth it is sharply tilted to the left from the main body axis at an angle of 20°–25°. In lateral outline the dorsal surface of the body appears only slightly convex, while the ventral surface is almost plane. Posteriorly, both surfaces become strongly convex and terminate respectively in rounded dorsal and ventral lobes. Both lateral surfaces are moderately convex, and together with the small, rounded operculum contribute to the ovoidal appearance of the animal in dorsal view. The lateral surfaces gradually taper posteriorly toward the caudal lobes. Two conspicuous lobes, of great diagnostic importance, are on the posterior end. The dorsal lobe is slightly over half as long as the ventral one (0.17 as compared to 0.31 of the dorso-ventral diameter in length) and varies in appearance from a small, straight hump to a relatively large, ventrally curved lobe. Always larger, the ventral lobe is dorsally curved toward its posterior end, and slightly hollowed out on its left dorsal surface. The two lobes are separated by a deep concavity, and lie in the same dorso-ventral median plane.

A rod-like macronucleus (0.80–1.07 of the dorso-ventral diameter in length) lies along the dorsal mid-line, and possesses nearly the same diameter throughout its length. It is smoothly rounded on the extremities and the ventral surface is straight or very slightly concave. There are two slight concavities on its dorsal surface, one in the middle and one on the left anterior surface. A small ellipsoidal micronucleus lies in the middle dorsal concavity of the macronucleus, while the anterior contractile vacuole occupies the anterior one.

Two small, subequal contractile vacuoles lie within the ectoplasm, one close against the left antero-dorsal surface of the macronucleus and the other at the extreme posterior end of the same organelle. Each vacuole has a small excretory canal leading to the dorsal surface where it opens through an excretory pore.

The mouth opens into a long oesophagus, which slopes dorsally at an angle of 15° and laterally to the right at an angle of about 25°, terminating at the middle part of the body against the right surface. This organelle is a characteristic structure. It appears ladder-like because of numerous transverse oesophageal membranelles, forming a zone which extends from the adoral region to the end of the oesophagus.

An inconspicuous boundary layer weakly separates ectoplasm from endoplasm. It lies close to the lateral and ventral walls of the body, but dorsally it underlies the macronucleus. On the posterior end it leaves the outer part of the body and continues toward the origin of the rectum. Consequently, the ectoplasmic layer is very thick in the region of the caudal lobes.

A simple, undifferentiated, slightly dorso-ventrally depressed rectum arises at the termination of the boundary layer. The rectum is short and opens to the exterior through an inconspicuous anus, which lies at the base of the dorsal surface of the ventral lobe.

Food.—Feeds on bacteria and small flagellates.

Variations.—Only the caudal lobes of this species show variability. Their size varies independently with that of the body, but both retain about the same relationship to each other. The dorsal lobe intergrades from a small, straight hump to a larger, ventrally curved fleshy lobe. The ventral lobe, on the contrary, is always conspicuous. It curves dorsally toward its extremity, and shows variation in the relative sharpness of the tip. Typically, the end of the ventral lobe is rounded and fleshy, but intergradations from this condition to that in which a rather sharp, spine-like contour is assumed, also occur. Similar variations were noted for this species by Dogiel (1927) for specimens taken from cattle and sheep from the U.S.S.R., Turkestan, and Siberia.

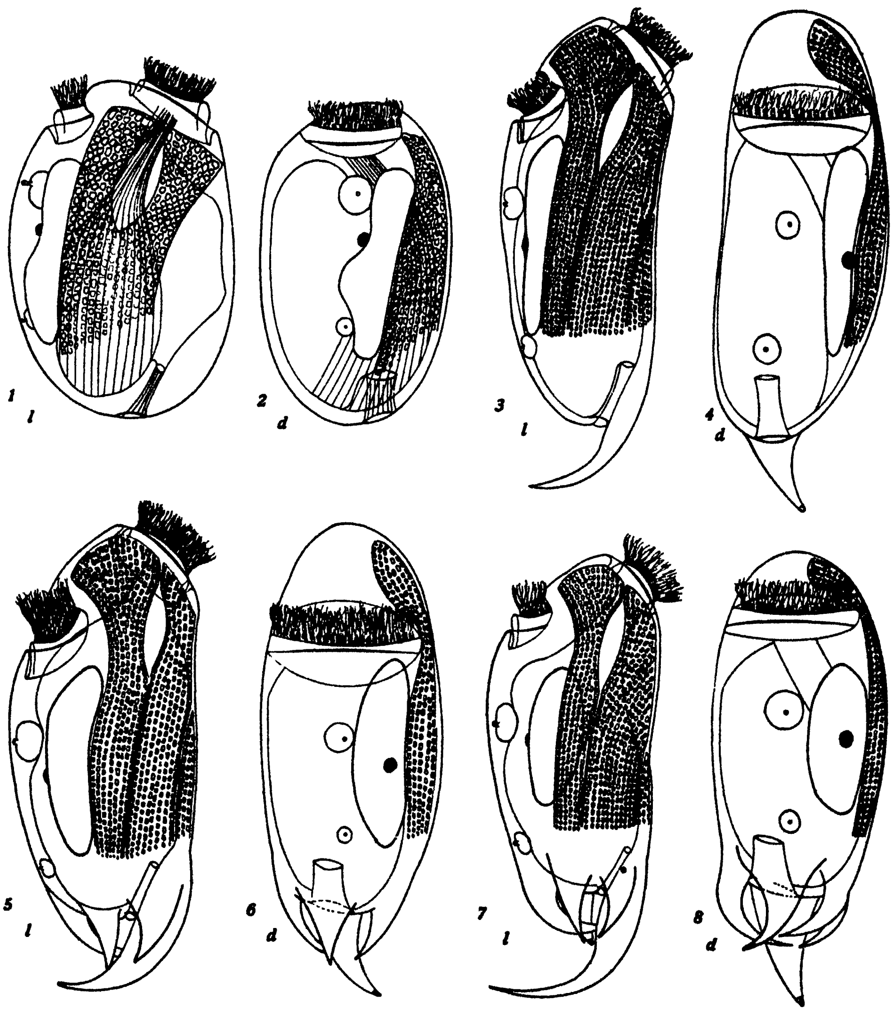

Fig. D. 1 and 2, *Metadinium rotundatum* sp. nov.; 3 and 4, *Epidinium caudatum* (Fiorentini 1889); 5 and 6, *E. quadricaudatum* (Sharp 1914); 7 and 8, *E. parvicaudatum* (Awerinzew and Mutafowa 1914). *l*, right lateral view; *d*, dorsal view. × 600.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 39(33–43)μ; transdiameter, 24(20–27)μ; dorso-ventral diameter, 29(25–32)μ; length of macronucleus, 27(24–31)μ; dorso-ventral diameter of mouth, 13(11–14)μ; length of dorsal lobe, 5(3–8)μ; length of ventral lobe, 9(7–12)μ.

Dogiel (1927) gives the following measurements for specimens of *E. bilobosum* from cattle and sheep from the U.S.S.R., Turkestan, and Siberia: length, 52(46–60)μ; dorso-ventral diameter, 36(30–44)μ; relation of length to dorso-ventral diameter, 1.4.

Occurrence.—Found in cattle and sheep from the U.S.S.R., Turkestan, and Siberia by Dogiel (1927) and in moderate numbers in *B. gaurus.*

Relationships.—*E. bilobosum* is closely related to *E. posterovesiculatum* and to *E. lobatum* through the position of the posterior contractile vacuole immediately behind the posterior end of the macronucleus. These three species constitute the *posterovesiculatum* group.

Epidinium caudatum (Fiorentini 1889)

Plate 27, figure 15; figure D, 3–4

Diagnosis.—See Kofoid and MacLennan (1933, p. 5).

Variations.—*E. caudatum* shows considerable variation in body length (2.00–2.95 dorso-ventral diameters in length), in the length of the macronucleus (1.05–1.55 dorso-ventral diameters in length), and in the length of the ventral caudal spine (0.37–0.72 of the dorso-ventral diameters in length).

Comparison with same species from B. indicus.—Specimens of *E. caudatum* from *B. gaurus* are smaller, 94(80–118)μ, than the corresponding specimens from *B. indicus,* 107(85–140)μ (Kofoid and MacLennan, 1933). A comparison of the tables of measurements for members of this species in the two hosts demonstrates very close similarities in all body proportions.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 94(80–118)μ; transdiameter, 37(30–45)μ; dorso-ventral diameter, 40(35–45)μ; length of macronucleus, 48(40–62)μ; dorso-ventral diameter of mouth, 18 (17–20)μ; length of spine, 21(13–26)μ.

Occurrence.—The wide geographical distribution and range of hosts were given by Kofoid and MacLennan (1933). It occurs also in moderate numbers in *B. gaurus.*

Epidinium quadricaudatum (Sharp 1914)

Plate 27, figure 16; figure D, 5–6

Diagnosis.—See Kofoid and MacLennan (1933, p. 10).

Comparison with specimens from B. indicus.—The single specimen of *E. quadricaudatum* from *B. gaurus* is significantly smaller, 88μ or 2.05 dorso-ventral diameters in length, than specimens from *B. indicus,* 116(110–119)μ or 2.29(2.27–2.32) dorso-ventral diameters in length (Kofoid and MacLennan, 1933). In body proportions and relative size of spines they are similar.

Measurements.—Based on 1 specimen found in *B. gaurus:* length, 88μ; transdiameter, 41μ; dorso-ventral diameter, 43μ; length of macronucleus, 42μ; dorso-ventral diameter of mouth, 20μ; length of ventral spine, 23μ.

Occurrence.—The limited distribution of *E. quadricaudatum* was given by Kofoid and MacLennan (1933). Only one specimen was found in *B. gaurus.*

Epidinium parvicaudatum (Awerinzew and Mutafowa 1914)

Plate 27, figure 17; figure D, 7–8

Diagnosis.—Identical with *E. caudatum* in all morphological features except spination; five caudal spines present—one large and ventral, one dorsal, one on the left side, and two on the right side. Length, 70–160μ; length of 8 specimens from *B. gaurus,* 92(70–120)μ.

Description.—The body of *E. parvicaudatum* is long, 2.14 (1.84–2.35) dorso-ventral diameters in length, relatively slender, and tapered posteriorly. It is almost circular in cross-section, being compressed laterally to only 0.95 (0.88–1.03) of the dorso-ventral diameter. Ventral and left surfaces are nearly plane or only slightly convex, and the ventral side is often concave in its anterior or middle third. The right and dorsal surfaces show greater convexity, especially in the mid-region of the body. The dorsal surface becomes nearly plane in the posterior third of the body, where it gives origin to the dorsal spine.

A moderately sized oral apparatus, 0.44 (0.43–0.47) of the dorso-ventral diameter in diameter, is tilted ventrally and laterally to the left at angles of 30°–40° from the main axis. The operculum is wide, smoothly rounded, and separates adoral from dorsal membranelle zones. The dorsal zone is set back on the dorsal surface about one-fifth of the body length from the anterior end, and encircles nearly one-third of the body.

Five caudal spines distinguish *E. parvicaudatum* from other members of the *E. ecaudatum* group. There is a large ventral spine, 0.67 (0.47–0.92) of the dorso-ventral diameter in length, with its extremity curved dorsally, corresponding to the single spine in *E. caudatum*. The second spine is dorsal in position, as is the second spine in *E. bicaudatum*. Two spines are present on the right surface, as in *E. quadricaudatum,* and an additional or fifth spine is present on the left side. The last four spines are only about half as long as the ventral spine, are subequal in length, and are all incurved at their extremities.

There are fine longitudinal striations over the cuticle. Three plates comprise the skeletal complex. They lie underneath the right surface of the body, and extend from the edge of the oral region posteriorly to the beginning of the last fourth of the body. The ventral and median plates lie in close proximity throughout their lengths. Dorsal and median plates, however, part in their anterior thirds but come together again at their anterior ends, leaving a double concave window of ectoplasm between. The plates follow the slight dextral spiral of the body, so that the anterior end of the ventral plate projects beneath the antero-ventral side of the body.

An elongated, rod-like macronucleus, 1.14 (0.84–1.37) dorso-ventral diameters in length, lies next to the right dorsal surface, adjacent to the dorsal edge of the skeletal complex. The small ellipsoidal micronucleus is found in a slight concavity in the mid-dorsal edge of the macronucleus.

Two contractile vacuoles, each with a conspicuous duct and pore, are on the dorsal surface, to the left of the macronucleus.

The oesophagus, marked weakly by fine fibrils, extends posteriorly and to the right from the oral region. From its point of origin near the beginning of the oesophagus the well-defined boundary layer of the endoplasm flares outward toward the body surfaces, but leaves a thin layer of ectoplasm as it extends to the posterior end. Its regularity is disturbed only where it forms concavities containing the vacuoles and the macronucleus. At the posterior end the boundary layer gives rise to a narrow, slightly dorso-ventrally depressed rectum, which extends postero-dorsally at an angle of about 30°. The anus lies at the base of the dorsal side of the ventral spine.

Food.—Consists of bacteria and small flagellates.

Variations.—Shows considerable variation in the length of the macronucleus (0.84–1.37 dorso-ventral diameters in length) and in the length of the large ventral spine (0.47–0.92 of the dorso-ventral diameter in length).

Measurements.—Based on 8 specimens found in *B. gaurus:* length, 92(70–120)μ; transdiameter, 41(37–47)μ; dorso-ventral diameter, 43(38–51)μ; length of macronucleus, 49(32–70)μ; dorso-ventral diameter of mouth, 19 (15–22)μ; length of ventral spine, 29(22–36)μ.

Occurrence.—In addition to the localities given by Kofoid and MacLennan (1933) this species occurs in limited numbers in *B. gaurus.*

Relationships.—*E. parvicaudatum* is the sixth and last member of the *E. ecaudatum* group, showing the greatest complexity in the caudal spination of all members of the series.

Eremoplastron rostratum (Fiorentini 1889)

Plate 28, figure 19; figure E, 1–12

Diagnosis.—See Kofoid and MacLennan (1932, p. 91).

Variations.—The caudal spine of *E. rostratum* shows variation in length and curvature (fig. E, 3–12). It is 0.44(0.31–0.58) of the dorso-ventral diameter in length, and this variability is entirely independent of variability in length of the body. All intergradations are found from specimens having the spine directed nearly straight posteriorly to those in which it is curved dorsally to lie close to the posterior surface of the body. Dogiel (1927) noted a similar condition in specimens from cattle from the U.S.S.R.

The vertical depth of the postero-dorsal ectoplasmic flange also exhibits a significant variability, 0.07–0.18 of the dorso-ventral diameter in depth, and ranges from a very small, inconspicuous elevation to a relatively large flange (fig. E, 3–12).

Comparison with same species from B. indicus.—Specimens of *E. rostratum* from *B. gaurus* differ from those from *B. indicus* in length of body, both absolute and relative, and in relative length of the caudal spine. *E. rostratum* taken from the gaur is 39(34–48)μ or 1.44(1.30–1.77) dorso-ventral diameters in length, and the caudal spine is 0.44(0.31–0.58) of the dorso-ventral diameter in length. Individuals from the Indian ox are 45(40–52)μ or 1.80(1.58–2.00) dorso-ventral diameters in length and the caudal spine is 0.60(0.46–0.96) of the dorso-ventral diameter in length (Kofoid and MacLennan, 1932). The most significant difference is that the specimens from the gaur are relatively shorter and stouter than the corresponding specimens from the Indian ox.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 39(34–48)μ; transdiameter, 24(22–27)μ; dorso-ventral diameter, 27(24–29)μ; length of macronucleus, 21(17–30)μ; dorso-ventral diameter of mouth, 11(10–13)μ; length of caudal spine, 12(9–15)μ; length of postero-dorsal flange, 4(2–5)μ.

Occurrence.—In addition to the localities and hosts given by Kofoid and MacLennan (1932) this species occurs in small numbers in *B. gaurus.*

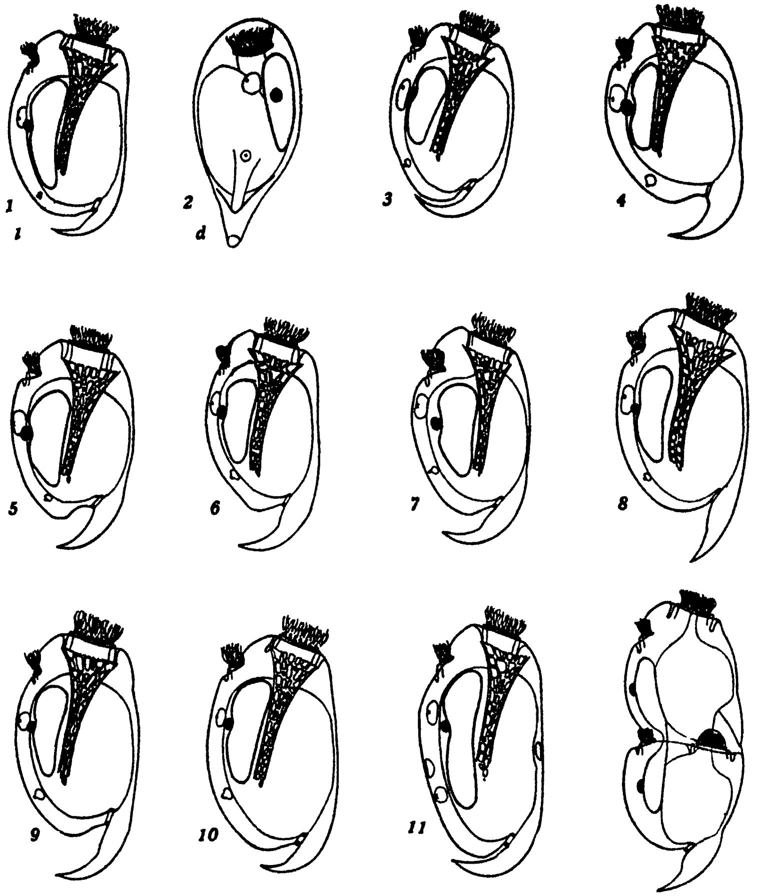

Fig. E. 1 and 2, *Eremoplastron rostratum* (Fiorentini 1889); 3–12, *E. rostratum* showing variability in body length, and in length and curvature of caudal spine. *l*, right lateral view; *d*, dorsal view. × 600.

Eudiplodinium maggii (Fiorentini 1889)

Plate 28, figure 20; figure F, 1–2

Diagnosis.—See Kofoid and MacLennan (1932, p. 105).

Variations.—*E. maggii* from *B. gaurus* does not vary significantly. This contrasts sharply with the variability in the cuticular groove and the skeletal plate in the same species from *B. indicus* (Kofoid and MacLennan, 1932).

Comparison with same species from B. indicus.—*E. maggii* from *B. gaurus* is significantly smaller, 102(80–117)μ or 1.42(1.33–1.51) dorso-ventral diameters in length, than the same species from *B. indicus*, 140(104–198)μ or 1.55(1.33–1.67) dorso-ventral diameters in length (Kofoid and MacLennan, 1932). Thus, the specimens from the gaur are not only absolutely smaller, but are relatively stouter, than those from the Indian ox. Consequently, the roughly triangular appearance of *E. maggii* from lateral view is partly obscured in specimens from *B. gaurus* because of this relative shortening of the body. It is also evident in comparing proportions, that the specimens from *B. gaurus* are more strongly compressed laterally, 0.78(0.58–0.90) as compared to 0 91(0.86–0.97) of the dorso-ventral diameter, and have relatively wider mouths, 0.42(0.35–0.45) as compared to 0.27(0.20–0.33) of the dorso-ventral diameter, than the corresponding specimens from *B. indicus*.

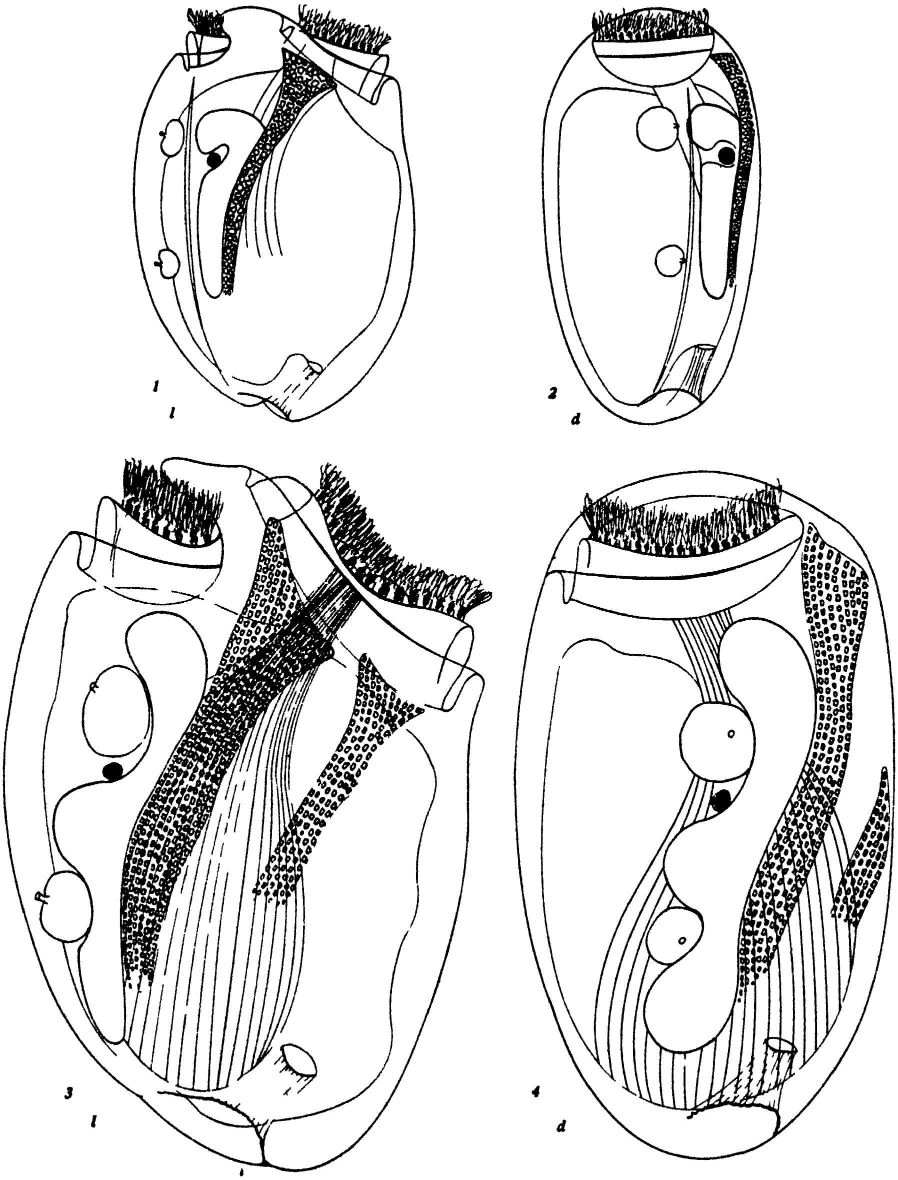

Fig. F. 1 and 2, *Eudiplodinium maggii* (Fiorentini 1889); 3 and 4, *Metadinium medium* Awerinzew and Mutafowa 1914. *l*, right lateral view; *d*, dorsal view. × 500.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 102(80–117)μ; transdiameter, 56(48–67)μ; dorso-ventral diameter, 72(58–86)μ;

length of macronucleus, 55(45–69)μ; dorso-ventral diameter of mouth, 30 (26–34)μ.

Occurrence.—*E. maggii* is another species of very wide geographical distribution. Recently it has been found in sheep from China by Hsiung (1931); in *B. indicus* from Colombo, Ceylon, and Coonoor, India, by Kofoid and MacLennan (1932); in cattle from Jugoslavia by Wertheim (1932); and in moderate numbers in *B. gaurus.*

Metadinium medium Awerinzew and Mutafowa 1914

Plate 29, figure 25; figure F, 3–4

Diagnosis.—See Kofoid and MacLennan (1932, p. 113).

Variations.—The typical body shape of *M. medium* varies considerably because it is often greatly distorted by large plant fibers which have been ingested. There are no other significant variations in morphological features in specimens from *B. gaurus.*

Comparison with same species from B. indicus.—The specimens of *M. medium* from *B. gaurus* are significantly smaller, 180(130–201)μ, than the corresponding specimens from *B. indicus,* 208(180–224)μ (Kofoid and MacLennan, 1932). The individuals from the gaur are further characterized by relatively wider mouth, 0.48 (0.41–0.57) of the dorso-ventral diameter as compared with 0.33(0.27–0.41) of the dorso-ventral diameter for the same structure in specimens from *B. indicus.* Other body proportions are strikingly similar in samples from these two hosts.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 180(130–201)μ; transdiameter, 102(80–124)μ; dorso-ventral diameter, 116(88–143)μ; length of macronucleus, 119(80–141)μ; dorso-ventral diameter of mouth, 56 (50–62)μ.

Occurrence.—*M. medium* also has a wide geographical distribution. It has been found recently in *B. indicus* from Colombo, Ceylon, and Coonoor, India, by Kofoid and MacLennan (1932) and occurs in limited numbers in *B. gaurus.*

Metadinium rotundatum sp. nov.

Plate 27, figure 18; figure D, 1–2

Diagnosis.—Body relatively short (1.43–1.55 dorso-ventral diameters in length), ovoid, and slightly laterally compressed; dorsal membranelle zone at about same level as adoral zone; skeletal complex of two plates underneath right surface, fused together at posterior ends; macronucleus elongate, with two shallow concavities on the left margin, one on each side of a large lateral lobe; oesophageal fibrils prominent, extending to posterior end of endoplasm; two contractile vacuoles along dorsal surface, one in each concavity of left side of macronucleus; rectum conspicuous, with faint longitudinal fibrils. Length of 10 specimens from *B. gaurus,* 65(52–73)μ.

Description.—*M. rotundatum* is the smallest member of this genus yet described. The body is relatively short, 1.48(1.43–1.55) dorso-ventral diameters in length, characteristically ovoid, and compressed laterally to 0.84(0.78–

0.97) of the dorso-ventral diameter. The dorsal surface is regularly convex throughout its entire length, while the ventral surface is most strongly convex in its posterior third. The right surface is a little more strongly convex than the left, which is often nearly plane in its middle third. All surfaces become strongly convex posteriorly where they terminate in an extremely smoothly rounded posterior end. Anteriorly, the right and left surfaces become strongly convex where they give rise to the shallow operculum, which is somewhat flattened on the left. Faint longitudinal striations cover the cuticle, which is relatively thick.

A well defined mouth, 0.45(0.39–0.51) of the dorso-ventral diameter in diameter, with conspicuous lips and furrows, occupies the ventral half of the anterior surface. It is tilted ventrally at an angle of about 15° and laterally to the left at an angle of about 25° from the main axis. The right side of the lip is continuous with the shallow operculum, which scarcely projects beyond the level of the adoral zone. The operculum distinctly separates the adoral zone from the dorsal zone of membranelles. The latter lies across the dorsal part of the anterior surface, either directly transverse or very slightly tilted to the left of the main body axis.

The skeletal complex of this species clearly relates it to *M. tauricum* (Dogiel and Fedorowa 1925), *M. ypsilon* (Dogiel 1925), and *M. magnum* (Dogiel 1925), all having two plates on the right side of the body which are fused posteriorly. In *M. rotundatum* the two plates lie underneath the right surface and are separate in their anterior halves. They fuse at about the level of the micronucleus so as to continue as one, terminating near the posterior end of the macronucleus. The dorsal side of these plates extends parallel with the ventral edge of the macronucleus, while the ventral plate extends obliquely postero-dorsally from the ventral part of the anterior end. The posterior end of the fused portion of the skeleton is also oblique, the dorsal side being most posterior. A double concave window of ectoplasm is left between the two plates in their anterior halves. There are 5–7 rows of prisms in each plate in the unfused anterior part.

The macronucleus is a characteristic feature of *M. rotundatum,* and might be compared to a reversed F with the top bar atrophied. It thus resembles the macronucleus of *M. ypsilon,* though the anterior lobe is not so well developed and is smoothly rounded instead of being truncated. It is elongated, 0.86 (0.75–0.93) of the dorso-ventral diameter in length, and possesses smoothly rounded ends. Ventral and right sides are plane or slightly concave. On the left edge, however, there is a relatively large, regular, lateral lobe, just posterior to the middle. There are two shallow lateral concavities on the same side, one between the large lobe and the anterior end, the other between the lobe and the posterior end. The anterior end is not greatly enlarged. The median lobe is only slightly perceptible in lateral view, the macronucleus appearing nearly rod-like. A small subspherical micronucleus lies in the anterior lateral concavity of the macronucleus, on its left margin.

Two contractile vacuoles, each with a conspicuous duct and pore, lie to the left of the macronucleus along the dorsal mid-line. The anterior vacuole is usually much larger than the posterior one, and is slightly anterior to the level of the micronucleus. The posterior vacuole lies in the posterior concavity of the left side of the macronucleus.

A small, tubular oesophagus extends posteriorly to the right from the mouth. At the beginning of the second third of the body it widens and opens up on its left side, and extends to the posterior end of the endoplasm underneath the right side. It is marked by deeply staining longitudinal fibrils, resembling the oesophagus of members of the genus *Ostracodinium.*

The boundary layer of the endoplasm extends from near the origin of the oesophagus to the posterior end of the body, clinging quite closely to the body surfaces. It is forced away from the dorsal surface by the macronucleus and vacuoles, which it underlies. The ectoplasm is thin in all parts except the regions ventral to the rectum, ventral to the oesophagus, and in the operculum. In the posterior end the endoplasmic sac narrows to give rise to the rectum.

The rectum is a well defined, slightly dorso-ventrally depressed tube, extending from the endoplasm postero-dorsally to the posterior end at an angle of about 25°. It is feebly marked by longitudinal fibrils. It terminates in an ellipsoidal anus which lies in the middle of the smoothly rounded posterior end.

Food.—Consists of pieces of plant debris.

Variations.—*M. rotundatum* is fairly constant in proportions, but varies considerably in actual body length.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 65(52–73)μ; transdiameter, 37(34–45)μ; dorso-ventral diameter, 44(35–51)μ; length of macronucleus, 38(30–45)μ; dorso-ventral diameter of mouth, 20(17–23)μ.

Occurrence.—*M. rotundatum* occurs in moderate numbers in *B. gaurus.*

Relationships.—*M. rotundatum* is most closely related to *M. ypsilon* (Dogiel 1925). It is similar to this species in having a skeletal complex of two plates fused posteriorly, a macronucleus of general similar shape, a similar position for the contractile vacuoles and the micronucleus, and a relatively thick cuticle. However, *M. rotundatum* is significantly smaller, has skeletal plates which are concave in their median unfused portions instead of parallel, and has a macronucleus with a rounded anterior end instead of an anterior end perpendicular to its long axis.

Ostracodinium mysorei sp. nov.

Plate 28, figure 23; figure G, 5–6, 9–12

Diagnosis.—Body short and relatively stout (1.16–1.60 dorso-ventral diameters in length); ventral and left surfaces slightly convex, right and dorsal surfaces more strongly convex; broad skeletal plate underneath right surface, wider in anterior third; macronucleus a straight, rod-like structure along the dorsal mid-line, gradually widening in anterior third; two subequal contractile vacuoles on left margin of macronucleus; one ventral caudal lobe of variable size, usually small; boundary layer forms posterior diverticulum dorsal to rectum. Length of 10 specimens from *B. gaurus,* 49(42–53)μ.

Description.—*O. mysorei* is small and relatively stout, 1.40(1.16–1.60) dorso-ventral diameters in length, and is strongly compressed laterally to 0.80

(0.72–0.93) of the dorso-ventral diameter. The ventral surface is almost plane or very slightly convex in its anterior two-thirds, but it becomes quite strongly convex in the posterior third where it merges into the ventral lobe. Likewise, the dorsal surface is more strongly convex in its posterior third, but the convexity of its anterior two-thirds is somewhat greater than that of the ventral surface. The relatively slight degree of convexity of these surfaces in their anterior two-thirds contributes to the somewhat rectangular appearance of the body in lateral view. In dorsal view the left side appears nearly plane, becoming moderately convex in the posterior third. The right surface is moderately convex, becoming strongly so in the anterior part where it is continuous with the prominent rounded operculum. Posteriorly, the right side becomes almost plane as it approaches the apex of the caudal lobe. These right and left surfaces are nearly parallel throughout the main body region, while they slope to form a triangular apex posteriorly, and merge into the rounded operculum anteriorly.

A moderately sized oral region, 0.40(0.31–0.44) of the dorso-ventral diameter in diameter, occupies the ventral part of the anterior face. The mouth is tilted ventrally and laterally to the left at angles of 20°–25° from the main axis. The outer adoral furrow is deep, but the lips are not well developed. The outer lip of the adoral zone on the right side extends continuously into the operculum, which is a prominent ectoplasmic protuberance extending slightly anteriorly from the level of the mouth. The dorsal membranelle zone lies at nearly the same transverse level as the adoral zone and is separated from it by the operculum. This zone encircles the antero-dorsal part of the body and is tilted only slightly to the left of the main body axis.

This species possesses a single ventral caudal lobe, 0.08(0.05–0.16) of the dorso-ventral diameter in length. In some specimens this lobe is almost too small to justify the designation as such, but in others it assumes larger proportions. The posterior end of the body dorsal to the anus is smoothly rounded, while the region ventral to the anus is enlarged into the caudal lobe. The lobe is formed by gradual tapering of the lateral and ventral surfaces of the body, and in dorsal view it appears as a blunt apex of a triangle.

A broad skeletal plate, consisting of about 20–30 longitudinal rows of polygonal prisms, lies underneath the right surface of the body. It extends from the level of the dorsal membranelle zone to near the posterior end of the macronucleus. Its dorsal visible edge is nearly straight and parallels the ventral edge of the macronucleus. Ventrally, however, the plate slopes obliquely in a curved plane antero-ventrally so as to project slightly underneath the antero-ventral edge of the body. The plate is therefore broadest in its anterior third.

The macronucleus is a straight, rod-like structure, 0.86(0.75–1.00) of the dorso-ventral diameter in length, with smoothly rounded ends, lying along the dorsal mid-line of the body. It is slightly dorso-ventrally depressed and in dorsal view is seen to broaden gradually anteriorly. A slight concavity on the left dorsal edge of the middle of the macronucleus contains the small subspherical micronucleus.

Two subequal contractile vacuoles are along the left edge of the macronucleus, one slightly anterior and the other slightly posterior to the level of the micronucleus. One, three, and even four vacuoles have also been observed, but the larger numbers occur only in elongated specimens which are approaching division. Each vacuole is connected to the dorsal surface of the body by a short duct and each opens through a small pore.

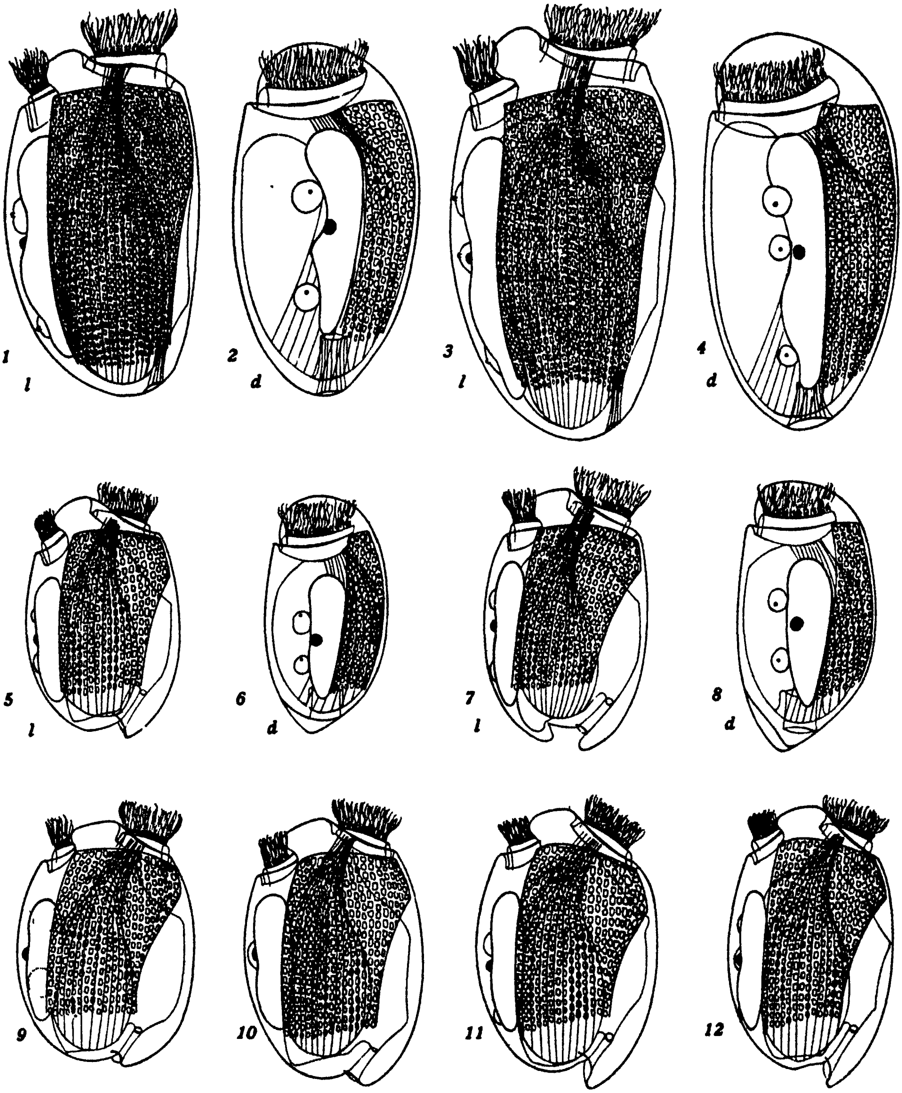

Fig. G. 1 and 2, *Ostracodinium gracile* (Dogiel 1925); 3 and 4, *O. trivesiculatum* Kofoid and MacLennan 1932; 5 and 6, *O. mysorei* sp. nov.; 7 and 8, *O. gauri* sp. nov.; 9–12, *O. mysorei*, showing variation in the size of the ventral caudal lobe. *l*, right lateral view; *d*, dorsal view. × 600.

The conspicuously fibrillated oesophagus extends as a narrow tube from the oral region posteriorly and to the right. At a point near the beginning of the second third of the body the fibrils suddenly spread out and extend along the right curvature of the body adjacent to the inner surface of the skeletal plate.

These fibrils terminate at the posterior end of the endoplasmic sac. The most dorsal of these fibrils extend underneath the macronucleus and end slightly to the left of the longitudinal mid-line. Thus, the oesophageal fibrils form a broad spoon beneath the skeletal plate. The tubular origin of the oesophagus forms the handle of the spoon and the concave part is directed inward and bounds the endoplasm.

An irregular boundary layer enclosing the endoplasm originates near the anterior end of the oesophagus. From its origin it flares outward toward the body wall where it extends to the posterior end. This layer is forced away from the body surfaces on the dorsal side, since it underlies the macronucleus and contractile vacuoles. In the posterior end the boundary layer forms a conspicuous diverticulum extending to the postero-dorsal surface of the body. Just dorsal to the anal region the boundary layer retreats from the posterior edge to give rise to the rectum. The ventral side of the layer extends irregularly along the ventral body surface directly to the origin of the rectum.

The rectum is conspicuous but undifferentiated. It is a strongly dorso-ventrally depressed tube extending from the endoplasm postero-dorsally to the posterior end at an angle of 30°–40°. It opens through a broad slit anus on the dorsal side of the base of the caudal lobe.

Food.—Small pieces of plant debris are found in the endoplasm.

Variations.—*O. mysorei* shows variation in the size of the ventral lobe and in the number of contractile vacuoles (fig. G, 5–6, 9–12). The ventral lobe intergrades from a small, rudimentary, pre-anal enlargement to a relatively prominent lobe. One to four contractile vacuoles are present, depending on the stage in the life-cycle at which the observations are made. Two appears to represent the typical number at the close of redifferentiation following binary fission.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 49(42–53)μ; transdiameter, 28(26–30)μ; dorso-ventral diameter, 35(32–40)μ; length of macronucleus, 30(26–37)μ; dorso-ventral diameter of mouth, 14(11–16)μ; length of lobe, 3(2–5)μ.

Occurrence.—*O. mysorei* occurs in moderate numbers in *B. gaurus.*

Relationships.—This species is related to *O. gauri* sp. nov. in all respects except caudal morphology. *O. mysorei* has only a single ventral lobe instead of two, as in *O. gauri.*

Ostracodinium gauri sp. nov.

Plate 28, figure 21; figure G, 7–8

Diagnosis.—Morphologically identical with *O. mysorei* in all respects except caudal armature; two caudal lobes of subequal size present—one ventral, the other dorsal. Length of 10 specimens from *B. gaurus,* 54(44–70)μ.

Description.—This species corresponds in all morphological features except caudal armature, and in size and proportions, to *O. mysorei* described above.

Two lobes are present on the posterior end of the body of *O. gauri.* One is ventral, 0.13(0.08–0.18) of the dorso-ventral diameter in length, and is formed by the continuation of the body convexities on its ventral surface, while dorsally it is slightly concave. The anal opening lies at the base of the dorsal surface of the ventral lobe. The second lobe is dorsal in position, is subequal with or slightly smaller than the ventral one, and shows no concavities or flattening. Both lobes are formed by gradual tapering of the surfaces of the body and in dorsal view they appear as blunt apices of triangles.

Variations.—*O. gauri* shows considerable variation in body length, size of caudal lobes, and in number of contractile vacuoles. Of these, variability in body length and vacuole number are correlated with division stages of the animals.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 54(44–70)μ; transdiameter, 30(23–40)μ; dorso-ventral diameter, 39(33–50)μ; length of macronucleus, 33(27–40)μ; dorso-ventral diameter of mouth, 14(12–17)μ; length of ventral lobe, 5(3–8)μ.

Occurrence.—*O. gauri* is found in moderate numbers in *B. gaurus.*

Relationships.—*O. gauri* is related to *O. mysorei* in body size, body shape, shape of macronucleus, shape of skeletal plate, posterior diverticulum of endoplasmic sac, and vacuole number. *O. gauri,* however, has two caudal lobes, while *O. mysorei* has only a small, ventral lobe.

O. gauri is related to *O. dilobum* Dogiel 1927 only in the possession of two caudal lobes. It differs from *O. dilobum* in being of smaller size, in having only two vacuoles instead of five, and in possessing a posterior diverticulum in the endoplasmic sac.

Ostracodinium gracile (Dogiel 1925)

Plate 28, figure 22; figure G, 1–2

Diagnosis.—See Kofoid and MacLennan (1932, p. 127).

Variations.—*O. gracile* maintains a high degree of constancy in general morphological features. In division stages, however, elongation of the body is often accompanied by the appearance of a third vacuole between the original two.

Comparison with same species from B. indicus.—Measurements demonstrate that specimens of *O. gracile* from *B. gaurus* are smaller and relatively shorter than the individuals of the same species from *B. indicus.* Thus *O. gracile* from the gaur is 75(60–85)μ or 1.70 (1.58–1.85) dorso-ventral diameters in length, while the corresponding specimens from the Indian ox are 101(92–125)μ or 1.88(1.75–2.16) dorso-ventral diameters in length (Kofoid and MacLennan, 1932). As a consequence of this difference, *O. gracile* from the gaur is relatively stout and partly loses the triangular appearance typical of the species.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 75(60–85)μ; transdiameter, 39(33–45)μ; dorso-ventral diameter, 44(39–47)μ; length of macronucleus, 48(40–57)μ; dorso-ventral diameter of mouth, 19(17–22)μ.

Occurrence.—In addition to the localities given by Kofoid and MacLennan (1932) *O. gracile* has been found by Hsiung (1931) in sheep from China, and it occurs in moderate numbers in *B. gaurus.*

Ostracodinium trivesiculatum Kofoid and MacLennan 1932

Plate 28, figure 24; figure G, 3–4

Diagnosis.—See Kofoid and MacLennan (1932, p. 129).

Variations.—Typically, *O. trivesiculatum* is roughly triangular in lateral view, but deviations from this condition to one of relative bluntness of the posterior end occur.

Comparison with same species from B. indicus.—*O. trivesiculatum* from *B. gaurus* is 82(72–91)μ or 1.78(1.70–2.02) dorso-ventral diameters in length, while the same species from the Indian ox is 98(78–100)μ or 1.94(1.67–2.34) dorso-ventral diameters in length (Kofoid and MacLennan, 1932). Therefore, the specimens from the two hosts differ absolutely and relatively, those from *B. gaurus* being shorter and relatively stouter.

Measurements.—Based on 10 specimens from *B. gaurus:* length, 82(72–91)μ; transdiameter, 41(36–43)μ; dorso-ventral diameter, 46(40–50)μ; length of macronucleus, 56(50–60)μ; dorso-ventral diameter of mouth, 20(17–22)μ.

Occurrence.—*O. trivesiculatum* was found in *B. indicus* from Coonoor, India, and Colombo, Ceylon, by Kofoid and MacLennan (1932) and in moderate numbers in *B. gaurus.*

OTHER PROTOZOA IN BOS GAURUS

THREE SPECIES of holotrichous ciliates are identified in the material from *Bos gaurus.* Of these *Dasytricha ruminantium* Schuberg 1888 is most abundant, *Isotricha prostoma* Stein 1859 is only moderately abundant, while *I. intestinalis* Stein 1858 is quite rare. *I. intestinalis* was not found in *B. indicus* by Kofoid and MacLennan (1933).

Great numbers of very small flagellates and a few amoebae are found in the material from *B. gaurus.* These are important as food for some of the Ophryoscolecidae.

DISCUSSION

THE CILIATES of the family Ophryoscolecidae, which inhabit the rumen and reticulum of the stomach of ruminants, exhibit diverse and numerous evolutionary tendencies. From a monophyletic origin, assumed because of the remarkable similarity in the basic structural plan of all the derived species, these Protozoa have developed into a large, diversified group consisting of 17 genera and 168 species now known. They have developed a greater complexity in food taking and locomotor organelles, a diversity of nuclear patterns, an increase in the number of contractile vacuoles, a progressive increase in the number of caudal projections and the complexity of the skeletal apparatus, and many have assumed relatively large body size. In sharp contrast to the usual degenerative evolution in parasitic animals, these ciliates have attained a high degree of structural complexity. Aside from the evolution of useful structures such as membranelles, contractile vacuoles, and skeletal system for storage of glycogen, these Protozoa also demonstrate progressive evolution of seemingly functionless structures such as caudal spines. This orthogenetic tendency is shown, for example, in the *Diplodinium anacanthum* group, which has species showing a graded increase in the number of caudal spines up to six, and in the *Epidinium ecaudatum* group, in which the series terminates with the species having five spines. The Ophryoscolecidae therefore exhibit many interesting problems in evolution.

The pronounced evolutionary progress of this group suggests that during the evolution of the host the physiological properties of the ruminant stomach also evolved significantly, exerting effects on the morphology of the ciliates. On this assumption, the stomach fauna evolved in close relationship with the evolution of the host. This assumption, assuming further that there were ample opportunities for cross-infection of the hosts before wide dispersal, readily accounts for the wide distribution of certain species of ciliates, while others, presumably of more recent origin, are limited to definite hosts or geographical areas. Such a view suggests the possibility of an affirmative answer to Becker's (1932) question concerning the extent to which the host specificity might be dependent upon physiological variability between species of hosts.

In the present study significant quantitative differences have been found to exist between individuals of the same species of ciliate from *Bos gaurus* and *B. indicus,* the latter host having been thoroughly studied by Kofoid and MacLennan (1930, 1932, 1933). As Dogiel (1927) has suggested, differences between individuals of the same species occurring in different hosts or in different geographical areas are sufficient to justify their designation as "races." These differences are explainable on the same basis as the evolution of the entire group, that is, by physiological peculiarities between the hosts. Whether these physiological differences between *B. gaurus* and *B. indicus* are individual, or truly specific, or ascribable to geographical isolation or to differences in kind and amount of host food, does not alter the fact that the differences do exist between the ciliates common to these two hosts.

The factor of isolation of the two hosts cannot be overlooked as a possible explanation of the differences between the species of ciliates common to both. Though both *B. gaurus* and *B. indicus* occur in India, there has been presumably no opportunity for cross-infection for a long period of time. *B. gaurus* is a wild species, never domesticated, restricting its activities to Indian jungles and wooded areas in the mountains. *B. indicus,* on the contrary, has been domesticated for centuries, and is essentially associated with civilization. It is possible that this seemingly complete isolation has functioned in the production of the quantitative differences between the species of ciliates common to the two hosts.

This study has also suggested the possible rôle of the food of the host as an immediate or slow cause of the quantitative differences between the ciliates. The gaur from which the specimens were obtained was taken just prior to the Indian monsoon, at a time when the jungle herbage was relatively very dry and scanty. It is conjectural to what degree this paucity of food of *B. gaurus* might have exerted an effect upon the reduction in size of the individual ciliates taken from the stomach at this season. It is also possible that the permanent diet of the jungle-dwelling gaur has been sufficiently different from that of the domesticated Indian ox to have been a factor in the production of the differences in their stomach faunas.

If these differences in the size of the ciliates from *B. gaurus* and *B. indicus* are only temporarily induced through differences in diet they are obviously not heritable. If the ciliates from the gaur could be caused to assume the same general size as those from the Indian ox merely by placing the wild host on the food menu of the domesticated one, the differences in the ciliate faunas would be proved nongenetic. In place of experimentation on the two hosts involved in this study the same problem can be solved in any domestic ruminant by testing the effects of kind and amount of food materials on the mean size of the ciliates of the stomach. This would detect any justification for designating these different size-groups as "races."

Should these size differences prove genetic by such experimentation, their explanation on the basis of natural selection is extremely doubtful since there is no obvious advantage to be gained in mere size differences. The size differences of the ciliates are no more explicable on this basis than the phenomenon of orthogenesis, which is well shown in the development of spines in the Ophryoscolecidae. All sizes of ciliates, armed or unarmed, seem equally well preserved.

SUMMARY

1. The material from one specimen of *Bos gaurus* from Mulehole, Mysore, India, contains twenty-five species of ciliates of the family Ophryoscolecidae, three species of holotrichous ciliates, and numerous flagellates and amoebae.

2. Eleven species of the Ophryoscolecidae from *B. gaurus* are compared with the same species from *B. indicus* (Kofoid and MacLennan 1930, 1932, 1933). The only differences observed between the ciliate faunas of these hosts are quantitative, the ciliates from the gaur being in general smaller and stouter than those from the Indian ox. It is suggested that these differences between the ciliate faunas of *B. gaurus* and *B. indicus* might be ascribable to physiological differences between hosts, to geographical isolation of hosts, or to differences in food habits of hosts.

3. Nine species of the Ophryoscolecidae not occurring in *B. indicus* are completely redescribed from *B. gaurus*.

4. Five new species of the Ophryoscolecidae are described from *B. gaurus*.

5. The ciliates from the wild relict member of the ox tribe, *B. gaurus,* show no primitive characteristics, being as complex in their evolutionary tendencies as those from *B. indicus* or other domesticated ruminants.

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EXPLANATION OF PLATES

All figures are from whole mounts stained with chlor-zinc-iodide and drawn with camera lucida, × 850.

PLATE 25

Fig. 1. *Entodinium acutonucleatum* Kofoid and MacLennan 1930.

Fig. 2. *Entodinium indicum* Kofoid and MacLennan 1930.

Fig. 3. *Entodinium longinucleatum* Dogiel 1925.

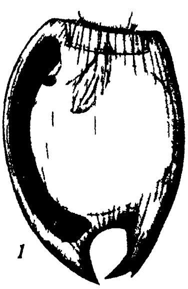
Fig. 4. *Entodinium contractum* sp. nov.

Fig. 5. *Entodinium curtum* sp. nov.

Fig. 6. *Entodinium nanellum* Dogiel 1922.

Fig. 7. *Eodinium bilobosum* (Dogiel 1927).

Fig. 8. *Diplodinium minor* Dogiel 1925.



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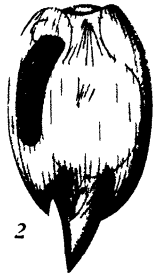

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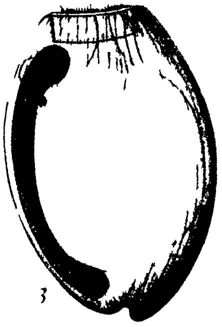

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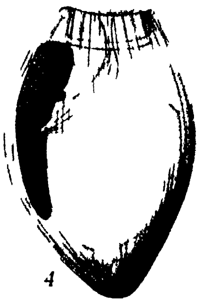

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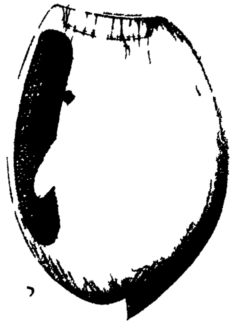

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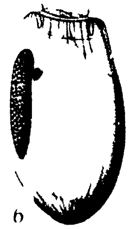

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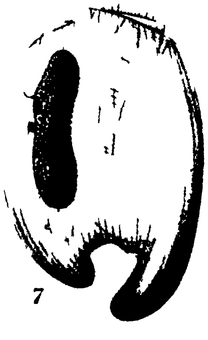

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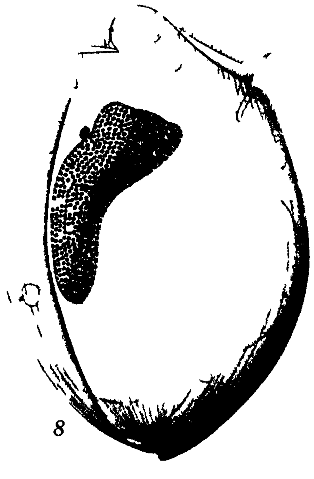

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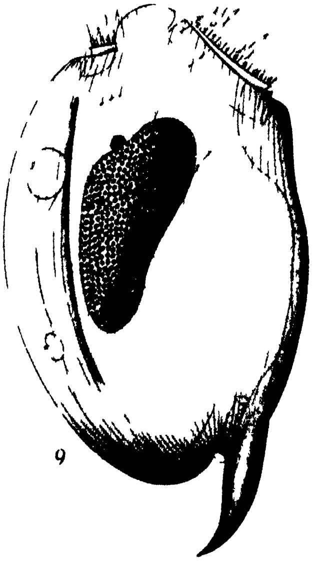
PLATE 26

Fig. 9. *Diplodinium monacanthum* Dogiel 1927.
Fig. 10. *Diplodinium diacanthum* Dogiel 1927.
Fig. 11. *Diplodinium triacanthum* Dogiel 1927.
Fig. 12. *Diplodinium tetracanthum* Dogiel 1927.
Fig. 13. *Diplodinium pentacanthum* Dogiel 1927.
Fig. 14. *Diplodinium anisacanthum* Cunha 1914.



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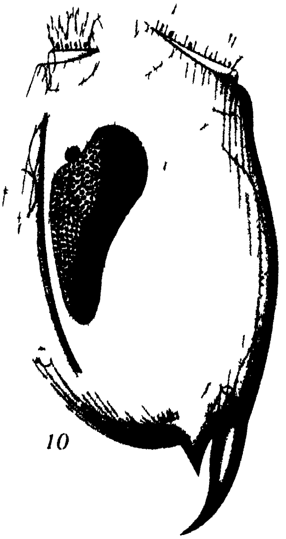

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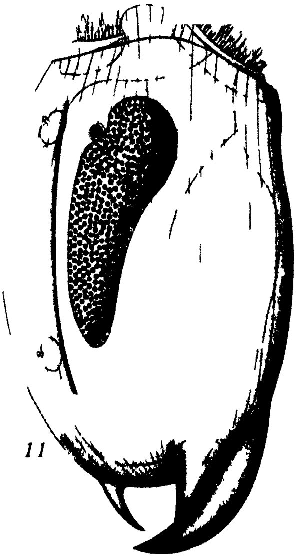

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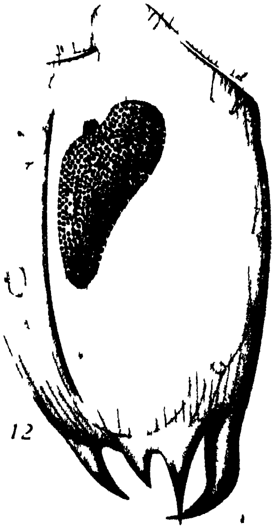

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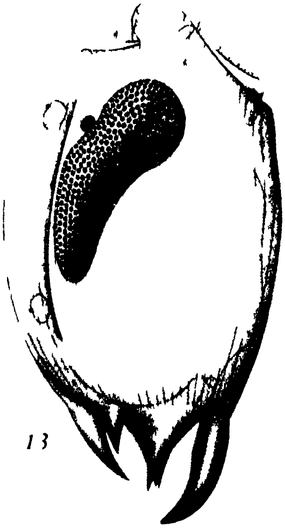

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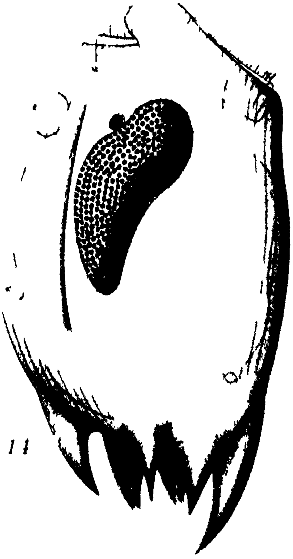

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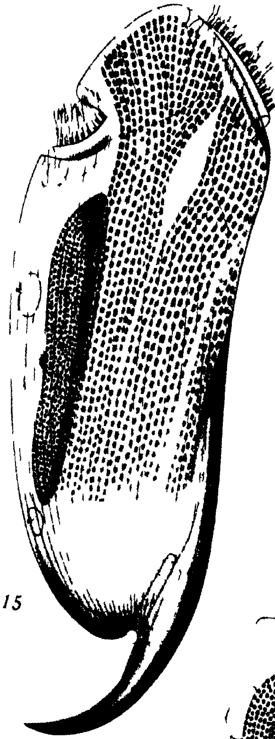
PLATE 27

Fig. 15. *Epidinium caudatum* (Fiorentini 1889).

Fig. 16. *Epidinium quadricaudatum* (Sharp 1914).

Fig. 17. *Epidinium parvicaudatum* (Awerinzew and Mutafowa 1914).

Fig. 18. *Metadinium rotundatum* sp. nov.



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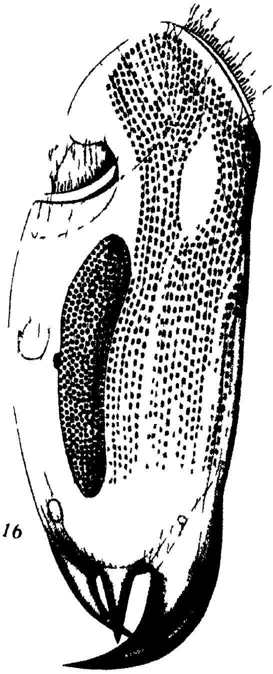

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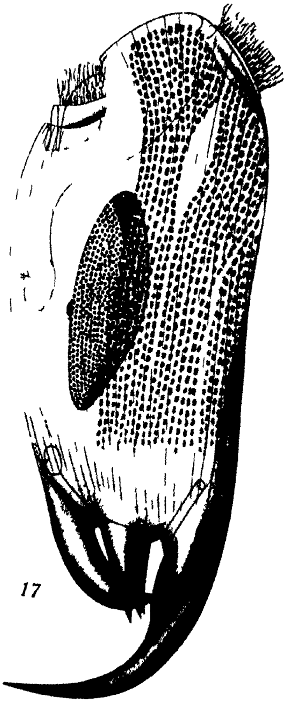

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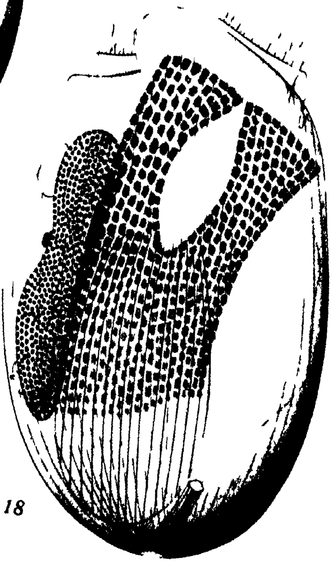

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PLATE 28

Fig. 19. *Eremoplastron rostratum* (Fiorentini 1889).

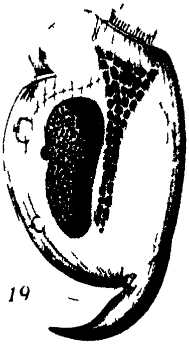
Fig. 20. *Eudiplodinium maggii* (Fiorentini 1889).

Fig. 21. *Ostracodinium gauri* sp. nov.

Fig. 22. *Ostracodinium gracile* (Dogiel 1925).

Fig. 23. *Ostracodinium mysorei* sp. nov.

Fig. 24. *Ostracodinium trivesiculatum* Kofoid and MacLennan 1932.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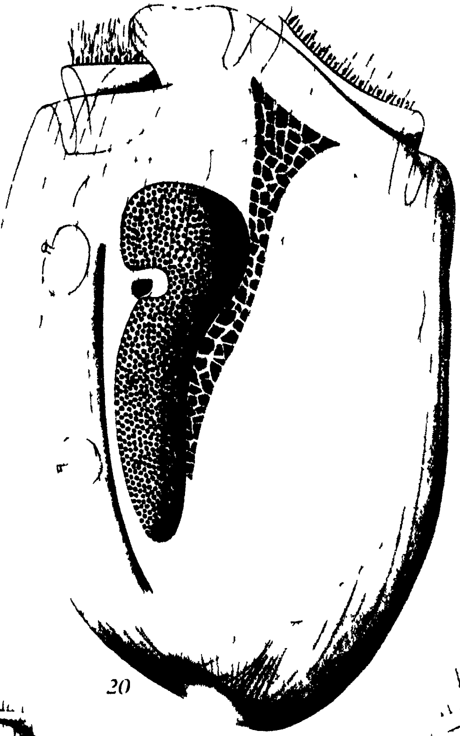
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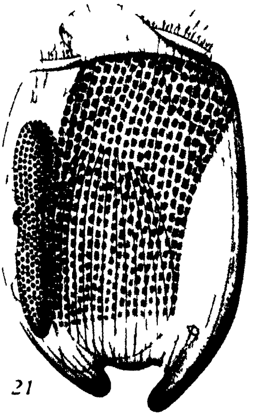
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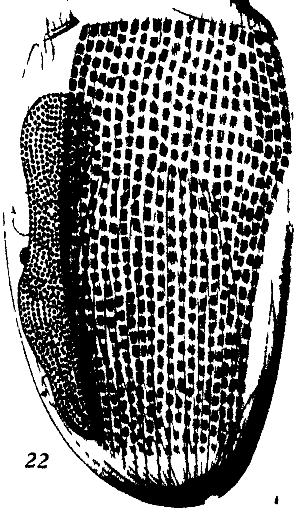
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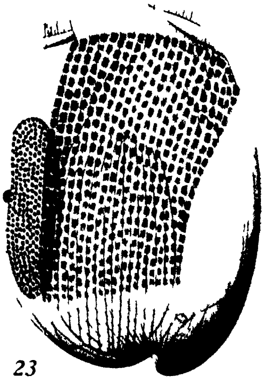
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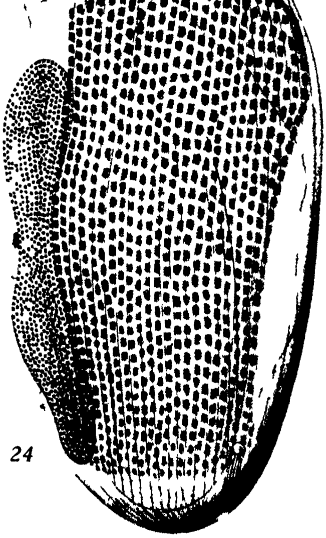
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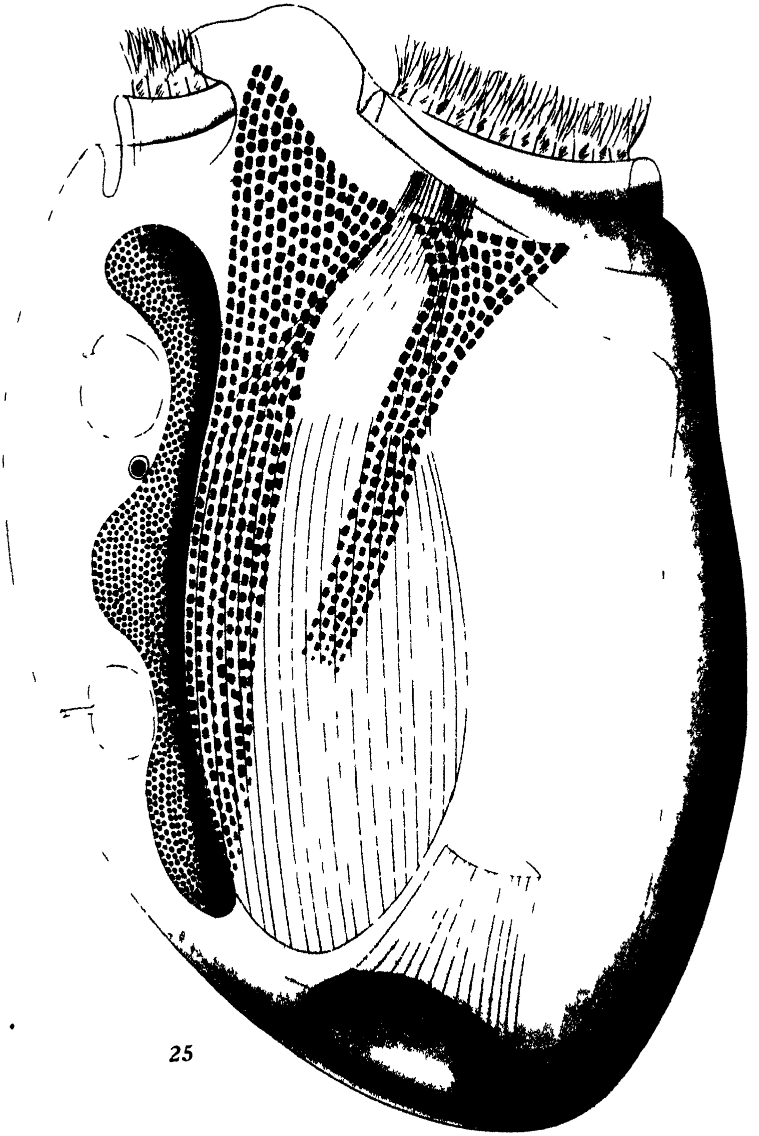
PLATE 29

Fig. 25. *Metadinium medium* Awerinzew and Mutafowa 1914.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THE DETERMINATION OF THE MEDULLARY PLATE IN TRITURUS TOROSUS (RATHKE)

BY

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INTRODUCTION

IN 1924 Spemann and H. Mangold found that the dorsal blastoporal lip of the early gastrula, when it comes into contact with tissue destined to form epidermis, changes the fate of this tissue. Instead of developing into part of the integument, the presumptive epidermis now develops into a central nervous system. Because of this apparently directive capacity exerted by the dorsal blastoporal lip, Spemann and H. Mangold termed it the organizer (*Organizator*).

This was the beginning of a number of investigations which laid the basis for what may be termed the organizer theory of medullary plate determination. According to the theory, presumptive medullary anlage is a neutral field so far as concerns the capacity to form nervous tissue; the normal medullary plate arises only after contact with the organizer.

The organizer theory has not gone unchallenged. The essential point of all rival theories has been that at least some degree of self-differentiative capacity is present in the presumptive medullary anlage *independent* of the effects exerted by the organizer.

Brachet (1923, 1927*a* and *b*), on the basis of his experiments involving the production of localized defects by means of a hot needle, as well as on the basis of transplantation experiments, has upheld the theory of the early determination of all the main organ-forming materials in certain Anura. Brachet's conclusions have not been supported by the work of Bytinski-Salz (1929*a*) so far as their applicability to the presumptive medullary material is concerned. Pieces of presumptive medullary material of *Rana temporaria* or *Hyla arborea,* implanted into the blastocoele of *Triton taeniatus* gastrulae, did not develop into medullary plate although the presumptive mesoderm and epidermis did differentiate according to origin.

Pasteels (1932) attempted to show predetermined areas other than that of the organizer by producing lesions with a glass needle in eggs of *Bufo vulgaris* and *Bombinator pachypus,* thirty minutes and two to three hours after fertilization. Of the resulting embryos, 50 per cent were normal; the rest showed deficiencies such as weaker development of the neural tube on the side punctured. Pasteels interprets these results as indicative of predetermined areas other than the organizer, although he does not consider these areas to be irreversibly determined. Such an interpretation can hardly be extended, however, to Schmidt's constriction experiments on Anura (1933), in which it was shown that a normal embryo may be obtained after the removal of an entire half of the blastula, provided the organizer is not included in the part removed.

Bautzmann (1929) and Kusche (1929) cultured part of the presumptive ectoderm from late blastulae and early gastrulae in the eye cavity of older larvae ("Interplant" experiment, Dürken). These experiments complicated rather than simplified the problem. Bautzmann and Kusche showed that chorda and connective tissue developed from both the presumptive medullary and epidermal materials. Kusche obtained nervous as well as other tissues from the presumptive medullary material of *Amblystoma* [*Ambystoma*], although not from that of *Triton*.

The interplant experiments just mentioned for a time seemed unfavorable to the organizer theory. Their significance has been altered, however, as a result of the recent work of Holtfreter (1933*d*) in which it is shown that living and dead parts of larval and adult salamanders (*Triton*) as well as parts of reptiles, birds, mammals, and certain invertebrates, are capable of inducing neural and other organs. The results of Bautzmann (1929) and Kusche (1929) may therefore be interpreted, not as evidence for self-differentiative capacities, but as a demonstration of the inductive principles present in salamander larvae.

Data regarded as unfavorable to the organizer theory of medullary differentiation have been advanced by Goerttler (1925, 1926, 1927). In one experiment Goerttler placed a large defect to the left side of the blastopore in a *Pleurodeles* gastrula. When neurulation occurred, the medullary fold on the same side as the defect arose in an abnormal position, which was, however, approximately the same position as that which the presumptive medullary anlage occupies in the gastrula stage. In other eggs the supposedly complete extirpation of the organizer did not prevent the development of the medullary folds. This would indicate that medullary differentiation can take place when no organizing substrate is present. Experiments of this type led to the statement of the theory of double assurance (*doppelte Sicherung*) by Spemann and Geinitz (1927), in which the undisputed inductive capacities of the organizer are viewed as a safety mechanism which assures the development of the nervous system.

Goerttler (1927) considered that the causal antecedent of medullary differentiation was to be found in the characteristic movements which occur in this material. This was termed the theory of dynamic determination. It has subsequently been flatly denied by Holtfreter (1933*a*).

Still another type of experiments, namely, those involving tissue culture (explantation), have yielded results seemingly unfavorable to the organizer theory. These experiments are the special subject of the present paper; the data presented indicate that the results of tissue culture can no longer be regarded as incongrous with the organizer theory. Certain of the findings of Erdmann (1931), Holtfreter (1933*b*), and Schechtman (1934) are especially concerned.

Erdmann (1931) found that in *Amblystoma* pure presumptive medullary material of the blastula, isolated before it has been lined by the chordamesodermal substrate, could develop elongate cells resembling those of the medullary plate. Holtfreter (1933*b*) also observed elongate cells, but considers their nature problematical and further investigation necessary. Schechtman (1934) found that the presumptive medullary material of *Triturus torosus* develops elongate as well as cuboid cells, but pointed out that *the significance of the elongate cells for the organizer theory depends upon their actual identification as medullary cells.* This identification has up to the present depended mainly on the elongate shape of the cells; but, as will be demonstrated in the present paper, the operational procedure itself produces an elongation also of presumptive ectodermal cells which makes these cells morphologically similar to those of the medullary plate.

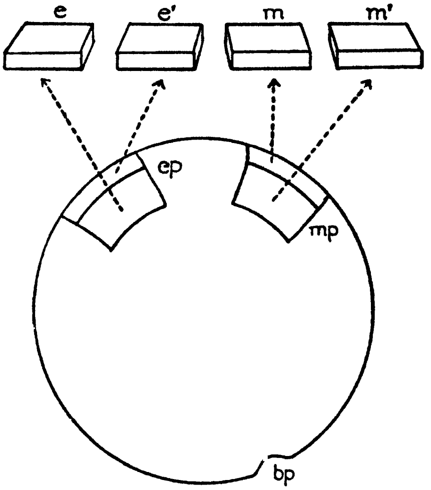

Fig. A. Schema representing the plan of operation. *bp.*, blastopore; *ep.*, presumptive epidermal explant; *e* and *e'* are explanted and fixed, respectively; *mp.*, presumptive medullary plate explant; *m* and *m'* are explanted and fixed, respectively.

MATERIALS AND METHODS

Plans of Operation

In order to evaluate the effects of explantation alone, as distinct from explantation followed by culture *in vitro*, the eggs from a single clutch were divided into two groups. The eggs of the first group were operated upon as shown in text figure A. The explants so prepared (*ep.*, or *mp.*) were fixed in Bouin's fluid several minutes after they were removed. The eggs of the second group, unoperated, were fixed *in toto* and pieces of fixed material were removed in the same way as shown in the figure. By this procedure it was possible to compare the histology of the tissue *in situ* and shortly after explantation.

The effects of culture *in vitro* on the histology of explants were observed by bisecting the explants (fig. A, *ep.* and *mp.*) just after their removal into two equal parts. One part (*e* and *m*) was cultured in Holtfreter's solution. The other part (*e'* and *m'*) was fixed as soon as possible after removal. It was thus possible to compare explants fixed shortly after their removal from the egg with those cultured *in vitro.*

Nomenclature

The types of cells observed during the course of the experiment are classified as cuboid, low columnar, columnar, flask-shaped, and elongate. The meanings attached to these terms are illustrated in figure B (p. 396).

CULTURE MEDIUM AND UTENSILS

All explants were prepared and maintained in Holtfreter's solution (1931). A single quantity of about twenty litres was prepared at the beginning of the experiment and used throughout. Operations were performed with a steel microknife and hair loop on a substrate of beeswax with the aid of a binocular microscope. Photographs were made with the Leitz "Macca" camera and are not retouched in any way.

THE MEDULLARY MATERIAL OF THE NEURULA

THE EXPLANTED MEDULLARY PLATE, NONCULTURED

IT IS OBVIOUS that a bit of tissue removed from its normal position in the embryo is thereafter subjected to a set of conditions differing from those encountered in the course of normal development. Under these circumstances one would hardly expect a fragment of medullary material to possess all the characteristics normally associated with medullary material. This applies especially to the morphological characteristics which are largely obliterated by the operation. A bit of medullary material observed soon after its removal from the embryo cannot usually be identified from a mere consideration of its external form. We must fall back upon histological examination. This reveals, we then observe, a compact mass of elongate cells with parallel elliptical nuclei. This point must be emphasized: that for the identification of explanted medullary material we must depend chiefly upon *histological* rather than morphological characteristics.

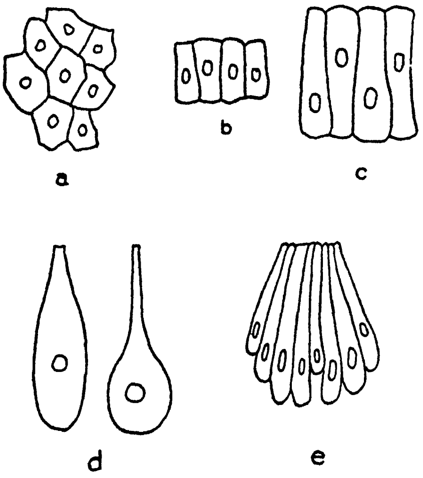

Fig. B. Schematic illustration of types of cells included under the terms cuboidal (*a*), low columnar (*b*), columnar (*c*), flask-shaped (*d*), and elongate (*e*).

THE EXPLANTED AND CULTURED MEDULLARY PLATE

The explants briefly considered above were not cultured. Culturing does not, however, appreciably alter their histological appearance. The explants are still characterized by the presence of a *compact mass* of elongate cells with elliptical nuclei. Two points should be emphasized concerning these elongate cells. First, they do not disappear in the period of culture; and secondly, they do not occur as single elements or in small groups, but are numerous and form a compact mass. These constitute our criteria of medullary differentiation.

Let us now examine more fully the effects of the operational procedure.

CHANGES OCCURRING IN EXPLANTS SOON AFTER EXPLANTATION

The Curling Behavior of Explants

It is well known to all who have operated on the amphibian embryo that certain tissues exhibit a marked tendency to curl after their extirpation. The fact that these tissues do not curl until removed from their normal positions in the embryo, indicates that certain tensions are present, and that these are brought into evidence by explantation. Two main types of behavior were observed. Some explants curl so that the pigmented outer side is concave; others, so that it is convex. Explants from the blastula characteristically curl *outward* (that is, toward the pigmented side) first and remain in this condition for a short time. Explants from the gastrula stage, however, commonly exhibit inward turning. The point to be emphasized in the present problem is the fact that a pronounced and often rapid curling of the explant occurs, which must involve differences in the relative tensions on the two sides of the ectoderm.

Curling plays an important rôle in producing the resemblance to medullary material which one finds shortly after explantation. It contributes to this resemblance in several ways. First, curling of the edges of the explant simulates the normal closure of the medullary plate to form a tube. Secondly, the pinching of the cells on one side produces the tapering condition observed in medullary cells. Thirdly, the concentration of the superficial pigment at various points, and its apparent extension into the cells produces a condition common to the normal medullary plate.

Postoperational Contraction of Explants

Postoperational contraction of explants may be demonstrated by comparing pieces fixed shortly after explantation with others fixed *in situ*. Thus, in the late blastula, the presumptive medullary and epidermal materials *in situ* are composed of cuboid, low columnar, and flask-shaped cells (figs. 1 and 4, pl. 30). Just after extirpation the explant tends to become thicker; its cells become longer and more slender as if subject to compression (see figs. 2 and 5, pl. 30). The nuclei become elongate and the superficial pigment becomes concentrated at various points. The resemblance to medullary material is increased by the curling of the explant, which tends to produce the tapering condition of the cells.

The mechanism involved in the curling of cell layers to become tubes remains a problem of great interest. Rhumbler (1902), Glaser (1914), and others have pointed out that the displacement of the cell contents toward one end of the cells or a greater absorption of water along one side of a layer of material could produce curling. As a result of these processes the cells should become roughly pyramidal in shape. But the cells of the medullary plate tend to become more *slender* as well as tapering, which must result from a contraction at right angles to the longer axes of the cells.

ELONGATION OF CELLS AT CUT EDGES OF EXPLANTS

It is usually after curling is well advanced that some of the cells at the cut edges of the explant take on an elongate form, as if tending to cover the exposed inner surface of the material (*a*, fig. C). A specific example may be described. A rectangular explant was removed from the presumptive medullary anlage of a late blastula at 11:55 A.M. Almost at once it curled outward so that the pigmented surface became concave.

At 12:04 P.M. the explant had flattened out.

At 12:06 the edges began to curl up in an inward direction, that is, tending to make the pigmented surface convex.

At 12:14 the explant had assumed the shape of a cup, the pigmented side constituting the outer surface.

At 12:37 the edges of the explant had met in most places and were apparently fusing. At the cut edges numerous columnar as well as flask-shaped and cuboidal cells were seen (*a*, fig. C). In the central part of the explant, that is, on that part of the surface farthest removed from the fusing edges, the cell outlines were mainly cuboidal (*b*, fig. C). The elongation of cells at cut surfaces provides another possible source for the medullary-like cells observed in explanted material.

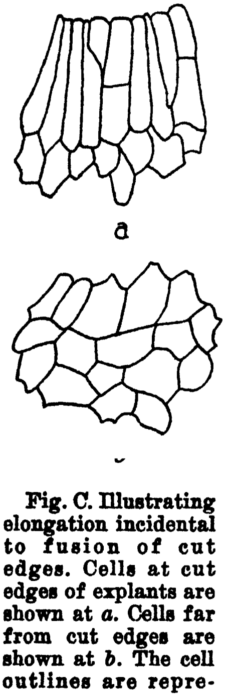

Fig. C. Illustrating elongation incidental to fusion of cut edges. Cells at cut edges of explants are shown at *a*. Cells far from cut edges are shown at *b*. The cell outlines are represented as seen from surface view in living material.

THE PRESUMPTIVE MEDULLARY MATERIAL OF THE MIDDLE AND LATE GASTRULAE

The term middle gastrula is here applied to stages beginning with the U-shaped blastopore, when the explants taken from the presumptive medullary material are commonly underbedded by archenteric roof. Explants taken at this stage have been subjected to the influences of the archenteric roof for varying lengths of time, according as they are derived from middle or from late gastrulae.

Before their removal from the egg, the cells comprising the presumptive medullary material of this stage are cuboidal, low columnar, or columnar (*a*, *b*, *c*, fig. B). Explantation is followed by changes illustrated in figure 2, plate 30. The elongation of the cells and their nuclei and the characteristic concentrations of superficial pigment recall common histological features of the medullary plate.

Maintenance of explants from the middle and late gastrulae *in vitro* has one of two effects, depending upon the length of time the presumptive medullary material has been underbedded by the archenteric roof. Explants from the *late* gastrula almost always produce the compact mass of elongate cells characteristic of the medullary plate. In explants from the middle gastrula typical medullary differentiation may be partly or completely absent, occasional elongate cells excepted.

THE PRESUMPTIVE MEDULLARY MATERIAL OF THE EARLY GASTRULA AND LATE BLASTULA

In these earlier stages of development the presumptive medullary anlage has not yet come into contact with the archenteric roof substrate. If the capacity to differentiate into medullary plate is dependent upon contact with the organizer, no medullary plate should be obtained from the late blastula and early gastrula.

In its normal position in the egg, the presumptive medullary material is composed of cuboid, low columnar, and columnar cells. A few flask-shaped cells can be discerned, the bulbous expanded part occurring in the inner of the two layers of cells, the slender neck part reaching up into the upper layer. There is no possibility of confusing this material with true medullary plate material.

If the explants are cultured *in vitro,* rather than fixed soon after their removal, a further change occurs. The numerous elongate cells which arise as a result of the surgical operation disappear to a large extent. But the disappearance is not complete, for occasional long cells may still be seen after a period of tissue culture (table 1, p. 402). This does not constitute typical medullary differentiation, for, as we have seen (p. 396), the typical histological picture is one of elongate cells in a compact mass.

The question is therefore narrowed down to the significance of the occasional elongate cells. The following results obtained with presumptive *epidermal* explants throw some light on this problem.

PRESUMPTIVE EPIDERMAL EXPLANTS

The presumptive epidermal material in normal development shows no capacity for development into medullary plate. It never comes into contact with the archenteric roof. Its development as an explant *in vitro* therefore gives us a picture of nonmedullary differentiation. How does this picture compare with that of the presumptive medullary material of the early gastrula?

In its normal position in the early gastrula the presumptive epidermis is composed of cells indistinguishable from those in the presumptive medullary region. Cuboid and low columnar cells predominate. As a result of its removal from the gastrula, the presumptive epidermal explant undergoes the same kind of curling and contractile changes already described for the presumptive medullary anlage. The similarities between the two anlagen continue even after a period of culture *in vitro,* for occasional elongate cells may be found in presumptive epidermal as well as in the medullary explant. In both, by far the greater number of the cells are of the "indifferent" cuboid type. We must therefore conclude that occasional elongate cells are not characteristic of the presumptive medullary region, but are common to the entire presumptive ectodermal field.

THE PERSISTENCE OF ELONGATE CELLS IN PRESUMPTIVE EPIDERMAL EXPLANTS

It has been pointed out above that occasional elongate cells occur in presumptive epidermal as well as presumptive medullary explants after a period of culture. In both, numerous elongate cells also arise just after the removal of the tissue from the egg. Is there a genetic relation between these? Are the occasional elongate cells simply a few persisting postoperational elongate cells?

METHOD

ALL THE EARLY GASTRULAE from a large clutch were removed from the gelatinous matrix and washed. From each egg a rectangular piece of tissue was removed from the presumptive medullary anlage and cut into two equal portions. The entire series of such pieces were then placed in one Stender dish of Holtfreter's solution. At successive intervals of time three of the explants were fixed and sectioned.

RESULTS

Explants fixed about five minutes after removal from the egg looked like medullary material. Within about one hour this resemblance had in large measure disappeared. Thereafter elongate, medullary-like cells occurred only as occasional elements. This would indicate that there is a genetic continuity between the post-operational elongate cells and the occasional elongate ones which are seen after a period of culture. Occasional elongate cells in presumptive medullary explants are not therefore of a medullary nature. They are created by the experimental procedure.

DISCUSSION

THE PRESUMPTIVE MEDULLARY MATERIAL gives no evidence of a capacity for medullary differentiation until it has come into contact with the subjacent organizer. This follows from the experiments described above. The occasional elongate cells are pseudomedullary. Making this allowance for the occasional elongate cells, explantation experiments are in full agreement with the organizer theory. They indicate that there is no more detectable capacity to form medullary plate in the presumptive medullary than in the presumptive epidermal material, before contact with the archenteric roof occurs.

The organizer theory has received further support from two recent and dissimilar experimental methods. Eakin (1933), by injecting a gelatine mass into the blastocoele, prevented the archenteric roof from coming into contact with a large part of the anterior medullary anlage, which he had stained with vital dye. In the gelatine-injected eggs the colored material showed no signs of medullary differentiation. In control eggs the material which had been similarly colored developed into medullary plate.

Holtfreter (1933*b*, *c*) effected the separation of the presumptive medullary material from the organizer in a different way. The egg membrane was removed and the naked eggs were kept in a dilute Ringer's solution. Both the presumptive epidermal and medullary materials may then be obtained as a mass of cells connected to the rest of the egg by a narrow thread of tissue. Isolated from the organizer in this manner, the presumptive medullary material shows no evidence of any capacity to form medullary plate.

Explantation experiments, the results obtained with exogastrulae (Holtfreter), experimental gastrulation (Eakin), and transplantation all agree in the verdict: Without the organizer there is no medullary plate.

SUMMARY

1. Explants from the presumptive medullary and epidermal regions of a urodele egg, *Triturus torosus* (Rathke), were grown *in vitro* in a salt solution.

2. It is shown that marked histological changes are produced in presumptive epidermal explants by the surgical operation. The cells become longer and more slender, and the curling of the entire explant tends to make the cells taper toward one end. The net result is that the cells take on a resemblance to medullary material.

3. The medullary-like cells thus produced in presumptive epidermal explants in large measure disappear in the period of culture. A few remain and these seem to be the occasional elongate cells regarded as medullary in nature.

4. Typical medullary differentiation is characterized by a compact mass of elongate cells rather than by occasional ones.

5. Such typical medullary differentiation occurs only in presumptive medullary explants which have come into contact with the archenteric roof (the organizer).

6. From all the foregoing it follows that the results of explantation experiments in *Triturus torosus* are in full agreement with the organizer theory of medullary plate determination.

ACKNOWLEDGMENTS

I wish to express my deep appreciation of the helpful advice and criticism of Professor J. Frank Daniel, under whose supervision this work was done.

TABLE 1

The Histological Appearance of Explants from the Presumptive Medullary Anlage of Successive Developmental Stages, Cultured in Vitro

Date of operation	Stage of donor	U*	Shapes of cells in explants	
			Fixed just after extirpation	Fixed† after culture *in vitro*
Jan. 12, 12 P.M.	Late blastula	–	Cuboid; few flask-shaped	Cuboid; low columnar
Jan. 13, 11 A.M.	Shallow blastopore	–	Flask-shaped; elongate	Low columnar; several elongate
		–	Cuboid (poor slide)	Low columnar; few elongate
Jan. 13, 5 P.M.	Crescentic blastopore	–	Cuboid; low columnar	Many elongate; many cuboid
		–	Cuboid; columnar; few flask-shaped	Low columnar; few elongate
Jan. 13, 10:30 P.M.	U-shaped blastopore	+	Columnar; many elongate	Cuboid; low columnar; few elongate
		–	Medullary	Cuboid; low columnar; few elongate
Jan. 14, 10 A.M.	Medium round yolk-plug	+	Medullary	Medullary in part; low columnar
		+	Medullary	Lost
Jan. 14, 1:30 P.M.	Medium round yolk-plug	+	Medullary	Medullary
			Medullary	Cuboid; columnar; few elongate
Jan. 14, 8:30 P.M.	Small yolk-plug	+	Medullary	Medullary
		+	Medullary	Medullary
Jan. 15, 9:40 A.M.	Earliest neurula	+	Medullary	Medullary
		+	Medullary	Medullary
Jan. 15, 5:30 P.M.	Sole-shaped medullary plate	+	Medullary	Medullary
		+	Medullary	Medullary

* U = presence (+) or absence (–) of archenteric roof under the explanted material.

† Date of fixation, January 19, 1934, 11 A.M.

TABLE 2

THE HISTOLOGICAL APPEARANCE OF EXPLANTS FROM THE PRESUMPTIVE EPIDERMAL ANLAGE OF SUCCESSIVE DEVELOPMENTAL STAGES. CAPSULE A

Date of operation	Stage of donor	Shapes of cells in explants	
		Fixed just after extirpation	Fixed* after culture *in vitro*
Jan. 12, 12 P.M.	Late blastula	Cuboid; blunt flask-shaped	Cuboid; flask; low columnar
Jan. 13, 11 A.M.	Shallow blastopore	Cuboid; few elongate	Lost
		Cuboid; few flask-shaped and elongate	Cuboid; low columnar; few elongate
Jan. 13, 5 P.M.	Crescentic blastopore	Cuboid; low columnar; few elongate; flask-shaped	Low columnar; few elongate
		Medullary	Cuboid; low columnar
Jan. 13, 10:30 P.M.	U-shaped blastopore	Medullary	Cuboid; low columnar; few elongate
		Medullary	Cuboid; columnar; few elongate
Jan. 14, 10 A.M.	Medium round yolk-plug	Medullary	Cuboid; flask-shaped
		Columnar; few elongate	Elongate
Jan. 14, 1:30 P.M.	Medium round yolk-plug	Medullary	Cuboid; columnar; few elongate
		Medullary	Cuboid; columnar; elongate
Jan. 14, 8:30 P.M.	Small yolk-plug	Medullary	Cuboid; few elongate
		Columnar; few elongate	Cuboid; few elongate
Jan. 15, 9:40 A.M.	Earliest neurual	Columnar	Cuboid; few flask-shaped
		Medullary	Cuboid; few elongate
Jan. 15, 5:30 P.M.	Sole-shaped medullary plate	Columnar; few elongate	Cuboid; few flask-shaped
		Medullary in part; columnar	Low columnar; cuboid

* Date of fixation, January 19, 1934, 11 A.M.

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EXPLANATION OF PLATE

Photographs not retouched in any way.

PLATE 30

Fig. 1. Presumptive medullary material from a late blastula, fixed *in situ*. No elongate cells are present.

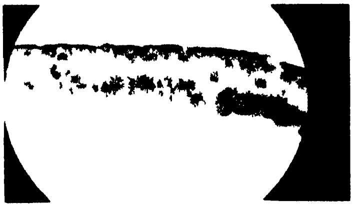
Fig. 2. Presumptive medullary material from a late blastula, fixed soon after extirpation. The explant has curled "outward." Note the longer, more slender cells, the concentrations of pigment and the "elliptical" nuclei.

Fig. 3. Presumptive medullary material from a late blastula, fixed after a period of culture *in vitro*. Note the several elongate cells, indicated chiefly by streaks of pigment in the photograph.

Fig. 4. Presumptive epidermal material from the late blastula, fixed *in situ*. No elongate cells are present.

Fig. 5. Presumptive epidermal material from the late blastula, fixed soon after extirpation. Note the lengthening of some of the cells, the concentrations of pigment, and the "elliptical" nuclei.

Fig. 6. Presumptive epidermal material from the late blastula, fixed after a period of culture *in vitro*. The majority of cells are roughly cuboidal in shape; several slender cells occur in the lower part of the explant.



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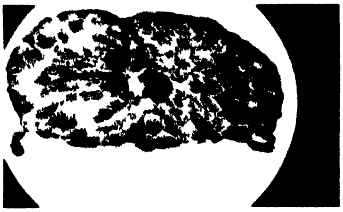

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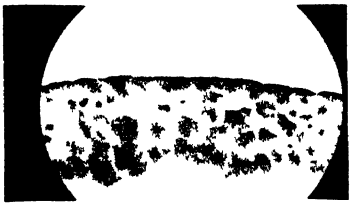

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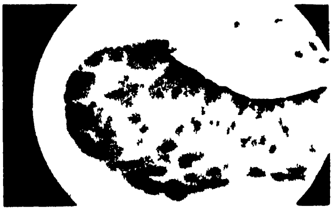

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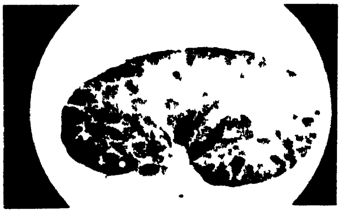

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ERRATUM

P. 80, l. 8 (l. 2 in explanation of figure A), *for* miosis *read* mitosis.